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Environment Canada
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Diets of Juvenile Atlantic Menhaden (*Brevoortia tyrannus*) in Three Estuarine Habitats as Determined from Fatty Acid Composition of Gut Contents

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Jeffries, H. P. 1975. Diets of juvenile Atlantic menhaden (*Brevoortia tyrannus*) in three estuarine habitats as determined from fatty acid composition of gut contents. J. Fish. Res. Board Can. 32: 587–592.

Juvenile Atlantic menhaden (*Brevoortia tyrannus*) feed on zooplankton and particulate organic matter, but the importance of each material in the diet cannot be visually determined, because food is ground to an amorphous paste in the fish's gizzard-like stomach. During early digestion in the anterior alimentary canal, fatty acids do not appear to change significantly, at least with respect to relative concentrations of saturated and unsaturated groups. Because zooplankton and particulate organic matter have markedly different fatty acid compositions, a hypothetical mixture of the two components can be calculated that best accounts for the observed fatty acid distributions of gut contents. Decreasing reliance on zooplankton, from bay through river to marsh, probably reflects resource abundances in three habitats and demonstrates adaptability of juvenile menhaden to different food supplies.

Jeffries, H. P. 1975. Diets of juvenile Atlantic menhaden (*Brevoortia tyrannus*) in three estuarine habitats as determined from fatty acid composition of gut contents. J. Fish. Res. Board Can. 32: 587–592.

Les jeunes de l'alose tyran de l'Atlantique (*Brevoortia tyrannus*) se nourissent de zooplancton et de matière organique particulaire. Il est cependant impossible de mesurer visuellement l'importance de chaque matériel dans le régime alimentaire, parce que la nourriture est réduite en pâte amorphe dans l'estomac, ressemblant à un gésier, du poisson. Au début de la digestion dans la partie antérieure du tube digestif, les acides gras ne semblent pas changer de façon significative, du moins en ce qui concerne les concentrations relatives des groupes saturés et insaturés. Comme le zooplancton et la matière organique particulaire ont des compositions en acides gras nettement différentes, on peut calculer un mélange hypothétique des deux composants qui correspond le mieux à la répartition observée des acides gras du contenu stomacal. Le fait que les jeunes aloses dépendent de moins en moins du zooplancton, en passant de la baie à la rivière et au marécage, reflète probablement des différences d'abondance de la ressource dans les trois habitats et est une preuve de l'adaptabilité des jeunes aloses à des sources de nourriture différentes.

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FISHERIES biologists rely on visual "gut content analyses" to determine prey of carnivorous species. In many filter feeders and benthic browsers, however, the contents are an unrecognizable mass. Surprisingly, a biochemical approach to this problem is all but unknown.

In a previous paper, fatty acids in gut contents and food items of marsh fishes were compared, revealing the relative importance of detritus in the diet (Jeffries 1972). The original method for calculating dietary mixtures from gut contents composition is simplified here and applied to juvenile menhaden, which range throughout estuarine habitats and feed both by filtering particulate organic material and by ingesting individual zooplankters.

Methods

Juvenile menhaden 40–60 mm long were captured with a beach seine at three locations in the Narragansett Bay, Rhode Island area: open bay (west shore of Prudence Island); polluted river (Edgewood in the Providence River, a tributary of Narragansett Bay); and tidal marsh (Bissel's Cove, bordering the bay's West Passage). The anterior alimentary canal was dissected, and about 2.5 g of partially digested food was obtained from 30 to 40 fish within 20 min of capture. The material obtained had a uniform consistency; it was freeze-dried immediately and stored at –20 C in a desiccator. Following saponification and methylation (Schultz and Quinn 1972) of triplicate 0.3 g portions, the fatty acid methyl esters were analyzed by gas chromatography on packed columns (diethylene glycol succinate, 18% on Chromosorb W-HP, 100/120 mesh).

Particulate organic matter from each habitat was filtered onto glass fibers, saponified, methylated, and analyzed for total fatty acid methyl esters by Dr David Schultz (Schultz 1974). Analytical agreement between our laboratories on a cod-liver oil sample was within a 5% range for major fatty acids with less than 20 carbon atoms.

Glass filters retained dissolved lipids in addition to phytoplankton and detritus (Schultz and Quinn 1972). Ideally, all components should have been separated and analyzed, but for present purposes it was believed that the retained fraction adequately represented the particulate organic matter available to filter feeding juvenile menhaden. Zooplankton samples taken previously in the open Bay were considered biochemically representative of the species available to juvenile menhaden in all three habitats (Jeffries 1964, 1970). Relative errors of major fatty acids (>10%) were less than ±5% for the menhaden samples and ±7% for the zooplankton.

Results

Gut contents of juvenile menhaden from all three habitats appeared similar by microscopic examination. Observed at 440×, about 95% of the material was amorphous; flagellated bacteria and fragments of planktonic organisms comprised the remainder.

Chemical patterns were intact, at least with respect to relative concentrations of the major fatty-acid groups. In material ingested by fish from the bay, the highly labile polyunsaturates (18:4, 20:5, and 22:6) had concentrations similar to those found in living plankton (Table 1,

TABLE 1. Fatty acid composition of materials removed from the alimentary canals of juvenile menhaden in the Narragansett Bay, R.I. area; weight percent of total fatty acid methyl esters; September–October 1971.

Fatty acid[a]	Bay	River	Marsh
12:0	0.6	1.1	0.9
12:1	0.3	0.6	0.2
13:0	0.4	0.4	0.2
B 14:0	0.3	0.5	0.3
14:0	3.7	6.1	11.5
14:1	0.0	1.6[b]	0.6
B 15:0	0.2		0.9
15:0	0.8	1.2[b]	2.9
15:1	0.8		0.6
B 16:0	1.4	1.5	0.8
16:0	21.1	28.0	37.9
16:1	5.2	6.5	11.1
16:3	0.5	nd[c]	0.4
17:0	2.3	2.4	1.4
17:1[d]	1.5	1.2	1.8
B 18:0	0.1	nd	nd
18:0	9.6	10.7	5.7
18:1	7.0	10.0	12.8
18:2	2.2	2.7	1.4
18:3	nd	nd	2.0[e]
18:4	5.5	0.8	1.9
19:0	0.8	0.5	0.7
20:0	0.5	0.4	0.6
20:1	0.8	1.4	nd
20:4	1.1	0.3	0.3
20:5	9.3	6.4	2.3
21:5	0.5	0.2	nd
22:0	1.4	0.9	0.3
22:1	0.9	1.5	0.5
22:4	0.3	tr[f]	nd
22:5	1.0	0.8	nd
22:6	19.6	11.9	nd
24:0	0.3	0.4	nd

[a]Shorthand designation for number of n carbon atoms: number of double bonds; B indicates branched-chain isomer (*iso* and *anteiso* forms).
[b]Not fully resolved.
[c]nd — not detected.
[d]Includes 16:1.
[e]Includes 20:1.
[f]tr — trace.

TABLE 2. Distributions of fatty acid groups[a] in observed and calulated diets of juvenile menhaden from three habitats; weight percents of total fatty acid methyl esters.

Habitat	Fatty acid group	Diet-percentages		Difference (observed–calculated)
		Observed	Calculated	
Bay	polyunsaturated	37.3	37.3	0
	unsaturated	19.2	27.4	−8.2
	saturated	43.5	35.3	+8.2
River	polyunsaturated	20.4	20.4	0
	unsaturated	25.1	35.2	−10.1
	saturated	54.5	44.4	+10.1
Marsh	polyunsaturated	4.5	4.5	0
	unsaturated	31.4	40.1	−8.7
	saturated	64.1	55.4	+8.7

[a]Chain lengths: double bonds — polyunsaturated, C18–22:4–6; unsaturated, C12–22:1–3; saturated, C12–24:0.

Jeffries 1970). These "marine acids" (Lovern 1964) decreased sharply in the gut contents of river and marsh fishes, 22:6, for example, dropping from 19.6% in the bay to absent in marsh fishes. Conversely, medium-chain saturated and monounsaturated acids increased through the series of habitats. Myristic acid (14:0) increased 3-fold, palmitoleic (16:1) 2-fold, and palmitic (16:0) by 80%. In Table 2, fatty acids in gut contents are grouped by unsaturation and chain length, summarizing a remarkably broad range of dietary change from one habitat to the next. From bay to marsh, the decrease in total polyunsaturates was almost 90%, whereas the generally shorter-chained saturates and unsaturates increased.

Compared with zooplankton, particulate organic matter was low in polyunsaturates and high in saturates (Table 3). Furthermore, the concentration of polyunsaturates in particulate organic matter decreased from bay to river and marsh, whereas the saturates increased.

TABLE 3. Distributions of fatty acid groups in retained (particulate) organic matter and zooplankton from the Narragansett Bay area; weight percents of total fatty acid methyl esters; µg fatty acid/mg dry wt.

Fatty acid group	Retained organic matter			Zooplankton
	Bay[a]	River[b]	Marsh[c]	Bay[d]
Polyunsaturated	12.2	0	0	38.9
Unsaturated	44.8	45.0	41.9	26.3
Saturated	43.0	55.0	58.1	34.8
µg/mg	10.1	25.6	4.9	65.4[e]

[a]Mean Oct.–Nov. 1972; Schultz (1974).
[b]Nov. 1972; Schultz (1974).
[c]Mean Sept.–Oct. 1972; Schultz and Quinn (1973).
[d]Sept. 1966; Jeffries (1970).
[e]Estimated, based on fatty acids comprising 60% of the total lipid.

Resource availabilities also differed between the three habitats. Going toward the marsh, the pattern was increased turbidity but less chlorophyll, indicating that rising amounts of suspended material were due to detritus and sediments swept from the bottom, not living phytoplankton (Table 4). Zooplankton was abundant in both the bay and, during fall at least, in the river. Characteristic of the marsh habitat was a scarcity of zooplankton amidst a heavy load of nonliving suspended material.

Assuming that zooplankton and particulate organic matter are the two major dietary components of juvenile menhaden, we can calculate a mixture of the two that would contain a relative concentration of total polyunsaturates equal to the concentration actually observed in gut contents. In this same food mixture, concentrations of total unsaturated and total saturated acids are then calculated and compared for agreement with observed values in gut contents. A hypothetical mixture is accepted if the correspondence of observed and predicted concentrations is within a reasonable estimate of pooled experimental errors. For each habitat, the calculated diet is from 8.2 to 10.1% too rich in unsaturated acids (Table 2), but even so, the mixture corresponds much closer to gut contents than either component (particulate organic matter or zooplankton) considered alone.

When weight percents of zooplankton and particulate organic matter corrected for their differences in absolute fatty acid concentrations were calculated, zooplankton comprised about ¾ of the diet in fish from the bay. However, in the marsh juvenile menhaden fed almost exclusively on par-

TABLE 4. Characteristics of the Narragansett Bay, R.I. area in terms of food resources for juvenile menhaden.

	Bay	River	Marsh
Turbidity[a]			
κ attenuation 1n/m	0.725	0.955	–
α attenuation 1n/m	2.516	2.723	–
Suspended matter mg/m³	2.9[b]	4.7[c]	22.3[e]
	2.6[b]	17.0[d]	9.5[f]
	4.0[g]		
Chlorophyll a μg/liter	60[h]	6[h]	9.5[i]
Zooplankton mg/m³, dry wt	109[h]	228[h]	–
	33[i]		
Animals/m³	49,000[j]	–	1–11,100[k]

[a]Schenck and Davis (1973); stations 7 and 15.
[b]Morton (1967) stations WP-1, WP-2, surface.
[c]Morton (1967); station PR-2.
[d]Nixon et al. (1973); 1.3 km from marina.
[e]Nixon et al. Mill Creek, nearby Bissel's Cove.
[f]Schultz and Quinn (1973); Bissel's Cove, average September and October, ebb tide.
[g]Calculated from Schultz (1974); station 4, average October and November.
[h]Nixon, unpublished; average September and October 1972; stations 4 and 7.
[i]Nixon, et al. (1973); average over an ebb tide, from Mill Creek, August.
[j]From Martin (1965); station 1 average September and October; Number 10 mesh net.
[k]Nixon and Oviatt (1973); range, June and July, No. 10 mesh net.

ticulate organic matter. The river, physiographically an intermediate habitat, is also an ecological intermediate (Table 5).

Discussion

FATTY ACIDS AS TRACERS OF TROPHIC RELATIONS

Fatty acid distributions in marine organisms have shown amazingly complex patterns, some sufficiently distinctive as to reveal feeding relations and environmental stress. Patterns fixed at a locus in the food web are both modified and passed on. Marine fatty acids are so metabolically active, however, that comparisons between tissues of living organisms may not reveal whether one is feeding on another. Analysis of gut contents, rather than tissue fatty acids, obviously represents a step bringing one closer to the problem.

TABLE 5. The diets of juvenile menhaden in three habitats; percent dry weight.

	Percent of diet	
Habitat	Zooplankton	Suspended organic matter (retained)
Bay	70.7	29.3
River	30.2	69.8
Marsh	1.0	99.0

In response to many suggestions for improving the method originally devised to estimate the importance of detritus in the diet of marsh fishes (Jeffries 1972), fatty acid groups have been redefined in the present paper from categories based on chromatographic retention to a simpler arrangement by chain length and unsaturation. Both approaches give similar results for the essentially nonparametric purpose intended. The previous grouping, nevertheless, may be less sensitive than the present arrangement to small changes in relative distribution of ingested fatty acids during early digestion. Differences between observed and calculated gut content compositions by the previous method range from 1.9 to 6.3%, a better agreement than the 8.2–10.1% error obtained with the new grouping (Table 2).

Four errors inherent to the method must be considered. First, dietary lipids are partially hydrolyzed during early digestion. It may be assumed, however, that all fatty acids are absorbed at equal rates in the anterior alimentary canal. The assumption appears reasonable (Hilditch and Williams 1964), but an experimentally derived correction would be required for more precise

determination than is intended here. Secondly, the method of back-calculating dietary ratios is based upon highly reactive polyunsaturates, chiefly 20:5 and 22:6. Although labile, these acids in gut contents of juvenile menhaden from the open bay are well represented, both acids in concentrations that are nearly identical to those found in *Acartia tonsa*, the dominant summer–fall copepod of Narragansett Bay (Jeffries 1970). Consequently, relative distribution appears adequately preserved for present purposes. Another drawback — rapid absorption of microbial fatty acids — would seem to be minimal, because bacteria and protozoans are not rich in the long-chain polyunsaturates. Referred to the fatty acid groups used here, microbes merely add unsaturates and medium-chain acids, not changing significantly the relative concentrations in the more refractory debris with which they are associated as the detrital fraction of particulate organic matter. Finally, the most important error may well be the inclusion of dissolved fatty acids adsorbed by the glass fiber filters that are used in the analysis of particulate organic matter. The adsorbed portion of retained organic matter is not available to animals, of course, but its pattern, as expressed by degrees of unsaturation, is probably the same as that of the particulate fraction. All limitations considered, the method seems more reliable for omnivorous fishes than visual examination of gut contents. Application to copepods and molluscs would require special sampling and microchemical procedures.

Growth and Diet of Menhaden

After menhaden spawn in coastal waters and bays, their larvae are transported into various estuarine habitats where they metamorphose into juveniles. Throughout late summer, juveniles grow rapidly before returning to the ocean in the fall as young adults. Larvae and recently metamorphosed menhaden feed by lunging at individual zooplankters, but by the time they reach 35 mm their grills and rakers have developed such that the adult's method of filtering particulate organic matter carried over gill surfaces is also possible (June and Carlson 1971). In late juvenile and adult stages, menhaden obtain zooplankton as well as phytoplankton by filtration (A. G. Durbin unpublished data).

Comparisons of fatty acids from food sources and gut contents confirm the different feeding modes of juveniles. When residing in a marsh, they use the adult's method, filtering particulate organic matter suspended in the turbid waters, but in open waters they may also prey on an abundance of individual zooplankters, using a method carried over from the larval stage.

Dry weight of zooplankton to particulate organic matter ingested by fish living in open waters of Narragansett Bay is 2.42:1; the ratio drops to 0.43:1 in the river habitat and to 0.01:1 in a nearby marsh, where much of the suspension is nonliving debris. The importance of suspended material to marsh inhabitants is shown by their behavior in a channel: they line up abreast of one another facing the tide, and the water turns from opaque to clear as it flows past their ranks.

In the river, reliance is also on suspended matter despite an abundant zooplankton crop, suggesting that, given adequate supplies of both components, juvenile menhaden favor filter feeding over predation. Generally, however, the diet's composition is directly related to resource availability throughout the estuarine environment.

Nutritional adequacy of the diet remains to be explored. An indication that marsh suspended matter by itself is an unsatisfactory food can be drawn from Nixon and Oviatt's (1973) measurements of caloric content in juvenile menhaden from the marsh also studied here. Marsh fish are significantly lower in calories than southern individuals, and their growth is retarded.

Estuaries are notably unstable in the kinds and amounts of resources produced. A species able to switch from one food source to another obviously compensates for resource variability. More than space is acquired: perhaps equally important is escape from predatory gamefish such as bluefish, *Pomatomus saltatrix*, which go on feeding rampages in open-water schools of juvenile menhaden. The menhaden's adaptability to several nursery areas must favor success in highly competitive situations.

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Toxaphene Effects on Growth and Bone Composition of Fathead Minnows, *Pimephales promelas*

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MEHRLE, P. M., AND F. L. MAYER JR. 1975. Toxaphene effects on growth and bone composition of fathead minnows, *Pimephales promelas*. J. Fish. Res. Board Can. 32: 593–598.

Fathead minnows (*Pimephales promelas*) were exposed to toxaphene (55–1230 ng/liter) in a flow-through diluter system for 150 days. Growth was not affected by toxaphene for up to 90 days of exposure, but within 150 days it was significantly reduced at all concentrations. Collagen content of the backbone was decreased ($P < 0.05$), amino acid composition was changed, and calcium concentration was increased. Results from this study suggest that toxaphene altered the development and quality of the backbone, and induced biochemical manifestations of the "broken-back" syndrome. Radiographic analyses of the fish support our hypothesis that toxaphene induced a weakened, fragile backbone.

MEHRLE, P. M., AND F. L. MAYER JR. 1975. Toxaphene effects on growth and bone composition of fathead minnows, *Pimephales promelas*. J. Fish. Res. Board Can. 32: 593–598.

Nous avons exposé pendant 150 jours des têtes-de-boule (*Pimephales promelas*) à du toxaphène (55–1230 ng/litre) dans un appareil à dilution avec courant d'eau. La croissance n'est pas affectée par une exposition allant jusuq'à 90 jours, mais elle est nettement ralentie en dedans de 150 jours à toutes les concentrations. Le contenu en collagène de la colonne vertébrale diminue ($P < 0.05$), la composition en acides aminés change, et la teneur en calcium augmente. Les résultats de cette étude suggèrent que le toxaphène affecte le développement et la qualité de la colonne vertébrale, et provoque les manifestations biochimique du syndrome « fracture de dos ». Des analyses radiographiques du poisson supportent notre hypothèse que le toxaphène est la cause d'une colonne vertébrale affaiblie et fragile.

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TOXAPHENE is an organochlorine insecticide used extensively by itself and in combination with other insecticides in agricultural areas of the United States. Approximately 30–40 million lb are applied annually on crops and livestock (Hercules Inc. 1970). Toxaphene is the technical grade of chlorinated camphene containing 67–69% chlorine and consists of approximately 175 isomers (Casida et al. 1974). The insecticide is a recognized contaminant in the aquatic environment based upon residues in fish ranging from 0.5 to 48 μg/g as reported by the National Pesticide Monitoring Program (B. L. Berger Branch of Environmental Improvement, Fish and Wildlife Service, Washington, D.C., personal communication). Only fish from the southern United States contained toxaphene residues and the residues most likely resulted from agriculture runoff. Nicholson et al. (1964) have reported seasonal water concentrations of toxaphene from 7 to 410 ng/liter (parts per trillion) in a stream from an agricultural section of Alabama. Terriere et al. (1966) found that toxaphene when used as a piscicide can persist in an oligotrophic lake up to 6 yr.

Although toxaphene is a very effective insecticide on target pests, its effects on nontarget organisms, such as fish, have not been thoroughly elucidated. Since toxaphene can be a persistent contaminant in the aquatic environment, we initiated studies to evaluate the effects of toxaphene on growth and backbone composition of fathead minnows (*Pimephales promelas*). This species represents an important family of forage species that are an essential link in the food economy of some natural ecosystems. They are also an important commercially reared bait fish.

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Materials and Methods

Ten-day-old fathead minnows were obtained from the National Water Quality Laboratory, Duluth, Minn., and were fed a commercial trout starter (EWOS). The commercial diet was supplemented daily with live brine shrimp nauplii for the first 30 days. Test fish were continuously exposed to toxaphene for 150 days in a proportional diluter system modeled after Mount and Brungs (1967) and with a dilution factor of 0.5 between the concentrations. Well water (pH = 7.2–7.4, alkalinity = 237 mg/liter, hardness = 272 mg/liter) was delivered to each of the tanks at a rate of 570 ml/min through the diluter system. The diluter system, with a chemical metering device after McAllister et al. (1972), was used to deliver five concentrations of toxaphene and a control. Flow-splitting chambers designed by Benoit and Puglisi (1973) were utilized to thoroughly mix and divide each toxaphene concentration for delivery to the duplicate exposure tanks. Toxaphene concentrations in the water of each tank were measured weekly by gas–liquid chromatography with a minimum detection limit of 10 ng/liter (Stalling and Huckins 1975), and were 0, 55, 132, 288, 621, and 1230 ng/liter. Acetone was used as the carrier solvent for toxaphene and did not exceed 0.7 ml/liter.

The 12 tanks used for exposing the fish to toxaphene measured 30.5 cm deep × 88.9 cm long × 30.5 cm wide and had a water depth of 20.3 cm. Each tank was divided into three compartments. One compartment was 61 cm long × 30.5 cm wide and the other two compartments were each 27.9 cm long × 15.2 cm wide. Water temperature in the exposure tanks was 24.5 (±0.5) C and artificial daylight was provided by the method of Drummond and Dawson (1970). The water temperature and photoperiod regime were those recommended for fathead minnow chronic toxicity tests by the U.S. Environmental Protection Agency (1972). An experimental use sample of toxaphene (X-16189-49) was furnished by Hercules, Inc. and was used throughout the study.

One hundred fish were placed randomly in the large compartments of each tank for growth, biochemical, and residue determinations. After 60 days of exposure, 16 fish from the large compartment were measured and placed in each of the small compartments to enable us to observe growth more closely. The fish retained for growth determinations were further reduced to eight per compartment at 90 days. Weights and total lengths of the fish for growth were measured after 30 (length only), 60, 90, and 150 days of exposure. Toxaphene residue analyses were performed on pooled samples of 6–18 fish from each concentration (Stalling and Huckins 1975).

After 150 days of exposure, eight fish from each concentration, except the fish exposed to the highest toxaphene concentration, in which excessive mortality occurred, were sampled for biochemical analyses of backbones. The backbone was removed from each fish, dried at 110 C for 2 h in a forced air oven, split into two fractions, and weighed. Collagen was isolated from one fraction, according to the method of Flanagan and Nichols (1962). The isolated collagen was weighed and subjected to hydrolysis at 115 C in 5 ml of 6 N HCl for 16 h. Quantitative amino acid analyses were performed on the protein hydrolysates by gas–liquid chromatography (Gehrke et al. 1968). The other bone fraction was subjected to hydrolysis at 115 C in 3 ml of 6 N HCl for 16 h. Phosphorus was determined on the hydrolysate by the Fiske and Subbarow (1925) method and calcium was determined using atomic absorption spectrophotometry. The precision of each method varied less than 3%, and the recovery from spiked samples was 95–99%.

Skin was removed from the ventral portion of eight fish up to the lateral line, and the scales and musculature were removed. The skin was dried at 115 C in a forced air oven for 2 h, weighed, and then hydrolyzed in 5 ml of 6 N HCl at 115 C for 16 h. Skin samples were analyzed for amino acids from fish in the 0, 55, and 621 ng/liter exposures. Quantitative amino acid analyses were performed by gas–liquid chromatography as above.

Six fish from each concentration were subjected to a sublethal electrical shock to cause contraction of the body musculature and thereby apply a stress to the skeletal structures. These fish were placed in an 80-liter aquarium equipped with an AC electrode. A 60-V stimulus was applied for approximately 1 s at three 20-s intervals, after which the fish were anesthetized in M.S. 222 and X-rayed. The radiographs were examined for alterations in vertebral structures.

A randomized block design with each concentration duplicated was utilized and all data were analyzed by analysis of variance. A multiple means comparison test (least significant difference, LSD) was used to determine significant differences among treatments (Snedecor 1965).

Results and Discussion

All concentrations of toxaphene significantly decreased ($P < 0.05$) fish growth within 150 days of exposure (Table 1). Growth, as determined by length and weight measurements, was not altered significantly prior to the 150-day exposure. Toxaphene residues in fathead minnows after 150 days of exposure were 5.9, 13, 22, and 52 μg/g in fish exposed to 55, 132, 288, and 621 ng/liter of toxaphene, respectively. Residues in fish exposed to 55 and 132 ng/liter of toxaphene are in the range reported in fish by the National Pesticide Monitoring Program (1974). These values ranged from 0.5 to 48 μg/g; the averaged value (±SD) was 8.7 (±16) μg/g.

The backbone composition as measured by collagen, calcium, and phosphorus concentrations, of fathead minnows was altered by toxaphene exposure (Table 2). The backbone consists of an organic matrix, minerals, and water. Approxi-

TABLE 1. Length and weight of fathead minnows (*Pimephales promelas*) as affected by continuous exposure to toxaphene for 150 days.

Days of exposure	Measurement	Toxaphene concentration (ng/liter) 0	55	132	288	621	*F* value[a]
60	length (mm)	30.4	30.2	30.6	30.1	30.0	0.34
	weight (g)	0.27	0.27	0.28	0.27	0.27	0.42
90	length (mm)	40.0	39.2	39.2	38.9	38.8	0.76
	weight (g)	0.68	0.66	0.64	0.63	0.62	0.52
150	length (mm)	48.6	46.2[b]	45.2[b]	46.6[b]	46.2[b]	2.44
	weight (g)	1.28	1.14[b]	1.01[b]	1.14[b]	1.04[b]	2.52

[a] $F_{.05} = 2.26$; $F_{.01} = 3.12$.
[b] Significantly different from controls ($LSD_{0.05}$).

TABLE 2. Collagen, calcium, and phosphorus concentrations in fathead minnow backbones as affected by 150 days of continuous exposure to toxaphene. Values expressed as percent of dried backbone and represent the average of eight observations.

Backbone constituent	Toxaphene concentration (ng/liter) 0	55	132	288	621	*F* value[a]	$LSD_{.05}$
Collagen	32.27	26.89[b]	22.92[b]	19.94[b]	22.39[b]	7.64	2.14
Calcium	11.81	15.10[b]	23.26[b]	20.84[b]	24.31[b]	3.27	2.85
Phosphorus	4.08	3.55	3.66	3.78	3.48	2.63	0.19

[a] $F_{0.05} = 2.64$; $F_{0.01} = 3.91$.
[b] Significantly different from controls ($P < 0.05$).

mately 90% of the organic matrix is a protein, collagen, whereas the remaining organics are mucopolysaccharides, mucoproteins, and lipids (Nusagens et al. 1972). Calcification and mineralization occur around and within the collagen fibrils during development and maturation of bone. The bulk of the mineral fraction consists of calcium, phosphate, and hydroxide crystals called hydroxyapatite [$Ca_{10}(OH)_2(PO_4)_6$]. We felt that determining the effects of toxaphene on collagen, calcium, and phosphorus metabolism in the backbone could give an indication of the effects of toxaphene on bone development and bone quality. Collagen was significantly decreased ($P < 0.05$) by toxaphene, while the amount of calcium in the backbone was significantly increased. The concentration of phosphorus in bone was not significantly changed by toxaphene, but there was a trend towards a decrease. The collagen:calcium ratio in the backbones of the control groups was 2.73, whereas the ratio was 1.78, 0.99, 0.96, and 0.92 in the groups receiving 55, 132, 288, and 621 ng/liter, respectively. Thus, the amount of calcium was increased relative to both the dry weight of bone and the organic matrix.

The biochemical analyses performed on the backbone and the length–weight measurements reflected the same effects of toxaphene after 150 days of exposure, i.e. growth rate and bone development were significantly altered. The "no-effect" concentration was less than 55 ng/liter for both measurements. The collagen and mineral analyses gave an indication of not only the status of growth and development, but also the quality of the bone. Because toxaphene caused a decrease in the collagen content of the backbone and a concomitant increase in calcium, we postulated that the backbone might be more fragile, and therefore less tolerant to stress. The results of subjecting fish to an electrical stimulus supported our postulation that toxaphene also altered the quality of the backbone. Of the six fish in each group subjected to the stress of electrical shock, the backbones of four fish from each group exposed to toxaphene were altered, whereas the backbones of control fish were not. The backbones were broken in several locations as determined by X-ray analyses (Fig. 1). The observations confirmed that toxaphene caused the backbone to be fragile and more susceptible to stress.

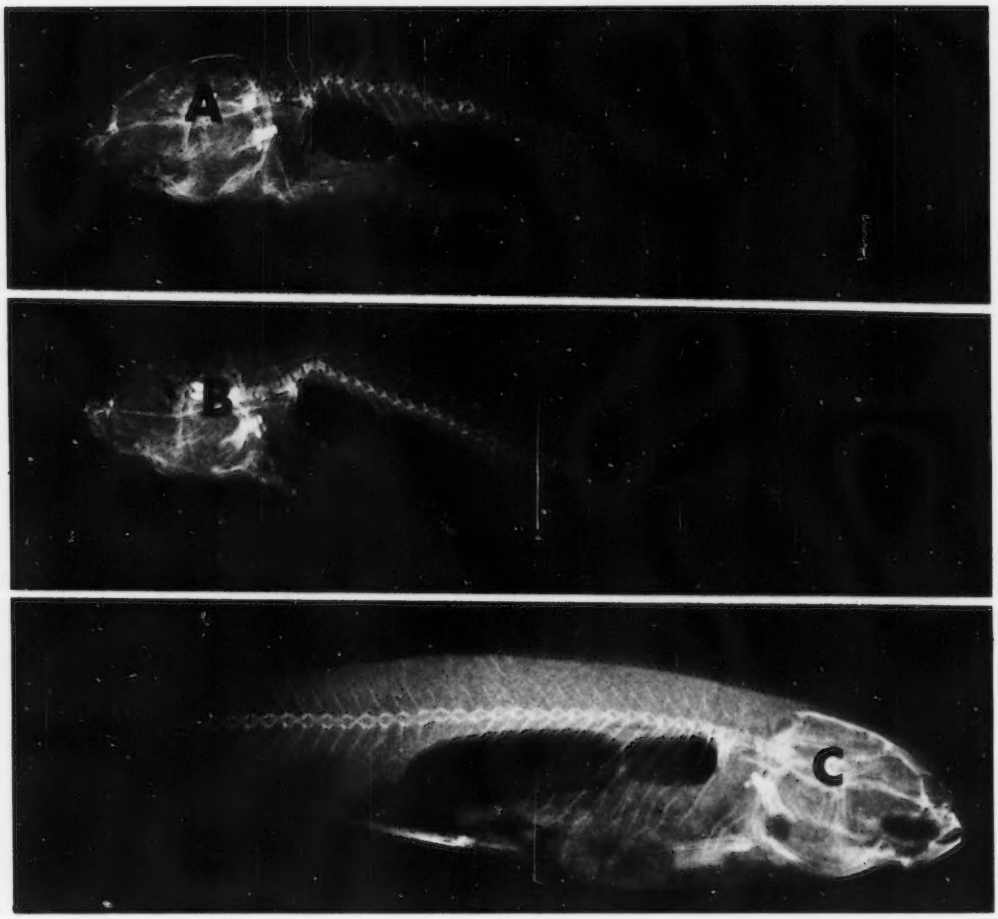

FIG. 1. Effects of toxaphene on structure of fathead minnow backbones. A and B are radiographs representative of fish exposed to 55 ng/liter of toxaphene and C is a control fish. Arrows point to areas of backbone affected.

The incidence of backbone anomalies has been reported in pond-cultured channel catfish by Sneed (1970). Three to five percent of the fish he examined exhibited symptoms such as fractured backbones, inability to coordinate swimming efforts, and the inability to maintain normal body position. Although the causes of this "broken-back" condition were not established, the possibilities of being nutritionally and pesticide-induced were considered.

The mechanism by which toxaphene induced the altered bone composition that rendered the backbone of fathead minnows more fragile is not completely understood. Several investigators have shown that both nutritional deficiencies and pesticides can induce a "broken-back" condition in fish. McCann and Jasper (1972) observed broken backs in bluegill within 4 h after exposure to acutely toxic concentrations of organophosphate insecticides. Backbone anomalies were observed at concentrations above and below the LC50 for six different organophosphate compounds. Dietary sources of vitamins D and C, phosphate, and calcium were found to be critical for proper bone development (Somogyi and Kodicek 1969). Wilson and Poe (1973) reported a decrease in

TABLE 3. Amino acid composition of backbone collagen in fathead minnows continuously exposed to toxaphene for 150 days. Values expressed as milligrams amino acid per gram of dried backbone collagen and represent the average of six observations.

Amino acid	Toxaphene concentration (ng/liter)					
	0	55	132	288	621	*F* value[a]
Alanine	22.9	20.6	22.2	26.0[b]	21.8	2.89
Valine	22.0	7.5[b]	12.5[b]	12.1[b]	5.9[b]	14.26
Glycine	153.8	150.5	152.8	158.6	156.6	2.12
Isoleucine	22.0	6.53[b]	11.4[b]	12.4[b]	5.1[b]	15.44
Leucine	36.7	15.9[b]	23.0	23.4[b]	12.1[b]	9.54
Proline	34.4	37.0	30.8	37.9	28.9	0.66
Threonine	14.5	17.6	16.2	17.9	16.4	1.19
Serine	12.6	14.3	11.5	14.1	11.0	0.45
Hydroxyproline	31.2	24.5[b]	14.2[b]	22.9[b]	26.5[b]	2.98
Methionine	2.3	2.2	2.6	2.6	2.0	0.49
Phenylalanine	25.0	15.3[b]	19.4[b]	18.7[b]	13.3	5.31
Aspartate	57.7	45.3	59.2	56.1	43.5	2.20
Glutamate	86.1	64.4	83.7	80.0	65.8	1.22
Tyrosine	2.3	2.4	2.1	2.5	3.4	0.62
Lysine	38.0	24.6[b]	25.4[b]	19.9[b]	13.0[b]	3.34

[a] $F_{0.05} = 2.71$; $F_{0.01} = 4.07$.
[b] Significantly different from controls ($LSD_{0.05}$).

collagen content of backbones and a broken back condition in channel catfish fed a vitamin C deficient diet. Hydroxyproline in the backbone collagen was significantly decreased. These investigators suggested the decrease in collagen caused the backbone to be more fragile. Halver et al. (1969) observed a similar condition in coho salmon and rainbow trout fed vitamin C deficient diets. In addition, wound repair mechanisms in the skin of these fish were delayed or inhibited due to the impaired collagen synthesis. Barnes et al. (1970) reported altered collagen formation in the skin of scorbutic guinea pigs. Also, Peterkofsky (1972) showed that vitamin C was required for collagen formation in chick embryo fibroblasts. The synthesized collagen in these fibroblasts was deficient in hydroxylated amino acids.

In our study the concentration of hydroxyproline, as well as seven other amino acids, were significantly decreased in collagen from the backbone of fish exposed to toxaphene (Table 3). However, based upon the amino acid content of collagen in the fish exposed to toxaphene as compared with the amino acid profile of collagen given by Wilson and Poe (1973), we believe that toxaphene could be altering several intermediary metabolic pathways associated with collagen and bone metabolism.

In addition, hydroxyproline in the skin was significantly decreased ($P < 0.05$) in fish exposed to the low and high concentrations of toxaphene. The hydroxyproline concentration in skin from the control group was 8.9 ± 0.9 mg/g ($\bar{x} \pm$ SE), whereas the concentrations were 7.2 ± 0.6 mg/g and 6.1 ± 0.5 mg/g in the groups exposed to 55 and 621 ng/liter, respectively. No other amino acid concentrations in the skin were altered by toxaphene. These results imply that toxaphene decreased the wound healing ability of skin, since collagen fibers in the skin are essential for wound repair. Also, these results suggest that toxaphene is not tissue specific in its effect on collagen; perhaps there is a generalized response by all collagen-containing tissue.

We suggest from these results that vitamin C metabolism may have been altered by toxaphene, which resulted in collagen synthesis being affected. Perhaps competition between structures or functions for vitamin C resulted in a functional deficiency of vitamin C. Wagstaff and Street (1971) showed that vitamin C was necessary for induction of microsomal hydroxylative enzymes which are responsible for detoxification of pesticides and other xenobiotics. Since utilization of vitamin C for detoxification of toxaphene was perhaps necessary for survival, a functional deficiency of vitamin C for development and maintenance of bone was induced. However, further study of the effects of toxaphene on bone development, de-

toxification mechanisms, and vitamin C are needed before definite conclusions can be made.

Since the whole-body residues of toxaphene from fish in the two lowest concentrations were in the range of those reported for toxaphene in fish sampled by the National Pesticide Monitoring Program, we suggest that results from the present study are realistic indications of the adverse effects of toxaphene on growth and bone development of fish in the environment. Since the "no-effect" concentration of toxaphene was less than our lowest concentration of 55 ng/liter, we feel that further investigations are needed to determine "no-effect" concentrations of toxaphene in fish.

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Adenosine Triphosphate Measurements in Soil and Marine Sediments

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A sulfuric acid–EDTA method for extracting ATP from soil and marine sediments was devised. The efficacy of this method is compared to the boiling Tris extraction procedure. Efficiency of ATP recovery from solid substrates typically ranged between 81–94% for the sulfuric acid–EDTA procedure in comparison to a recovery of 3–6% using boiling Tris buffer. The presence of various ions in the acid extract, however, inhibited light emission in the luciferin–luciferase measurement of ATP. Addition of EDTA to the extract reduced but did not eliminate the ionic interference. An internal ATP standard was thus used to determine the magnitude of the residual interference.

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Nous avons mis au point une méthode acide sulfurique–ATED pour l'extraction de l'ATP du sol et des sédiments marins. Nous comparons l'efficacité de cette méthode avec celle de l'extraction au Tris bouillant. L'efficacité de récupération de l'ATP des substrats solides varie typiquement de 81 à 94% par la méthode acide sulfurique–ATED, comparativement à 3–6% par celle du tampon Tris. La présence de divers ions dans l'extrait acide empêche toutefois l'émission de lumière lors de la mesure luciférine–luciférase de l'ATP. L'addition d'ATED à l'extrait réduit, mais n'élimine pas, l'interférence ionique. Nous avons donc utilisé un étalon ATP interne pour déterminer l'ordre de grandeur de l'interférence résiduaire.

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WITHIN recent years, adenosine triphosphate (ATP) has been shown to offer great promise for measuring microbial biomass. The reliability of ATP as a biomass indicator has been demonstrated by Holm-Hansen (1973a,b).

Extraction of ATP from the appropriate sample material is the first step in the ATP assay. Several ATP extraction procedures have been described and include the following: perchloric acid (Strange et al. 1963; Cole et al. 1967); trichloroacetic acid (Beutler and Mathai 1967); sulfuric acid (Lee et al. 1971); boiling glycine buffer (Lin and Cohen 1968); boiling water (Strehler 1968); dimethylsulfoxide (DMSO) in Tris buffer (Lee et al. 1971); *n*-butanol (Chappelle and Levin 1968); DMSO in sulfuric acid (Lee et al. 1971); *n*-bromo-succinimide and EDTA (MacLeod et al. 1969); boiling chloroform (Dhople and Hanks 1973); and boiling Tris-borate buffer (Beutler and Baluda 1964).

A method has been described for the quantitative extraction of ATP from seawater samples (Holm-Hansen and Booth 1966) using boiling Tris buffer. This technique has been successfully applied to water samples and bacterial suspensions (Holm-Hansen and Booth 1966; Hamilton and Holm-Hansen 1967). However, when applied to sediments, boiling Tris gives inconsistent results. Lee et al. (1971) and Ausmus (1973) have independently compiled a comparison of ATP extraction techniques for soil and sediments. In both studies it was concluded that nonheating acid extraction methods achieved better results in recovering ATP from solid substrates. In this publication we describe a nonheating ATP extraction method, the effect of anions and cations on inhibiting the luciferin–luciferase reaction, and how ionic inhibition may be overcome.

EXTRACTION OF ATP

Sediments for ATP analysis were subsampled from a dredge, piston core, or gravity core using sterile glass core tubes, 8 mm ID, and calibrated to contain 1 cc. Subsamples were taken in triplicate, transferred to 100 × 16 mm culture tubes, covered with aluminum foil, and stored on ice prior to extraction.

Extraction was done by adding 5 ml of 0.6 N

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H_2SO_4 (5 C) to each sediment sample, and mixing the tubes on a vortex mixer intermittently at 10-s intervals for a total of 60 s. Longer vortex mixing did not enhance recovery. The tubes were then allowed to settle for 10 min. An internal standard consisting of 1 ml of 100 ng/ml ATP was added to one of the triplicate samples, and 1 ml of Tris buffer to the remaining two samples. The sediments were vortex mixed for an additional 10 s and then centrifuged for 5 min at 1800 × *g*. After centrifugation, 4 ml of the supernatant were transferred to a 10-ml beaker, followed by addition of 1 ml of a 0.048 M (18 g/liter) solution of the disodium salt of ethylenedinitrilo-tetracetic acid (EDTA), made up in Tris. The pH of the acid extract was adjusted to 7.8 with NaOH using a Corning 476050 combination electrode. Tris has been reported to interfere with the accuracy of some electrodes (Sigma Chemical Company), but no effects were discernible at the molarity used. After neutralizing, the sample was quantitatively transferred to a calibrated test tube, the volume brought up to 10 ml with Tris and frozen for subsequent analysis.

In assaying the acid-extracted ATP samples, a standard curve prepared in Tris cannot be used to calculate final ATP concentrations. A series of ATP standards for acid-extracted samples was prepared at the same time the environmental samples were extracted. These standards were prepared by mixing 3 ml of 0.6 N H_2SO_4, 1 ml of 0.048 M EDTA solution and 1 ml of the desired ATP standard, neutralizing with NaOH and adding Tris for a final volume of 10 ml. The standards covered a range of 0.3–50 ng ATP/ml and were frozen along with the extracted samples to be assayed by the bioluminiscent reaction.

Boiling Tris extractions were used for comparative purposes in a number of experiments with *Escherichia coli* purchased from the American Type Culture Collection (ATCC 11775). The cells were either collected on a membrane filter (0.45 μm) under 10 psi vacuum or 0.1 ml of cell suspension was added directly to the Tris extractant. Sediments, sand, or bacteria-coated glass beads were put directly into the Tris. In all cases, the sample was added to 10 ml of boiling Tris buffer contained in a calibrated 25 mm OD screw cap tube. The Tris was maintained at 100 C by an oil bath at 120 C. The sample was boiled for 10 min in the Tris, cooled in an ice bath, and the volume restored to 10 ml with additional Tris. The extract was frozen until assayed. The Tris used in sample extraction and analysis was prepared as 0.1 M and stored in a refrigerator to prevent spoilage. Just before use, the Tris was diluted to 0.025 M.

In one experiment, glass beads were coated with bacteria to determine the effects of the solid phase on efficiency of ATP extraction. Resting cells of *E. coli* were harvested from nutrient agar plates and washed three times with sterile 0.85% NaCl. The suspension was adjusted to an optical density of 0.3 with sterile NaCl solution, yielding a final cell density of 2×10^8 cells/ml. One-half milliliter of the washed cell suspension was added to 2 g of 0.5 mm diameter glass beads, mixed, and allowed to air dry. The entire 2 g of bacteria-coated beads were then extracted using both the boiling Tris and the H_2SO_4 procedures.

The Bioluminescent Reaction and Its Measurement

Once extracted, ATP can readily be assayed by means of the firefly bioluminescent reaction (Strehler 1968). Firefly luciferin and luciferase react with ATP in the presence of Mg^{++} and molecular oxygen to yield a photon of light for each ATP molecule consumed at neutral or alkaline pH (McElroy et al. 1969). When the reactants are mixed, an initial burst of light occurs followed by a long decay period (Schram 1970). The addition of arsenate buffer to the reaction mixture retards the decay of luminescence after the initial light burst (Strehler and Totter 1954). Light emission in the luciferin–luciferase reaction is inhibited by the presence of both cations and anions (Aledort et al. 1966; Strehler 1968). Dilution of the enzyme mixture reduces the magnitude of the initial burst of light but does not affect the exponential relationship of the decay which lasts over a long period of time (Beutler and Baluda 1964; Rasmussen and Nielsen 1968; Schram 1970).

The luciferin–luciferase mixture used in our work was prepared by reconstituting each vial of lyophilized firefly lantern extract (Sigma Chemical Company, FLE-50) with 12.5 ml distilled water, 7.5 ml of 0.1 M arsenate buffer (Sigma Chemical Company, FF-AS-100), and 5 ml of 0.05 M magnesium sulfate. The enzyme was allowed to stand for between 6–7 h at 23–25 C in order to remove endogenous ATP. After aging, the preparation was filtered (Whatman #2) to remove residual debris. After clarification and during use, the enzyme was kept in an ice bath. One-half milliliter of enzyme was used for each ATP assay permitting about 45 assays at an approximate cost of 3–4 cents per assay.

A number of different light detecting instruments have been used for measuring the bioluminescent reaction. These generally can be divided into two types: those that measure the initial burst or peak intensity of the light emitted (Chappelle and Levin 1968; Rasmussen and Nielsen 1968; Allen 1973; Anderson and Davies 1973) and those that integrate the area under a portion of the decay curve that follows. A commercial instrument using peak intensity measurements has been developed but requires the use of specially prepared reagents (Allen 1973; E. I. DuPont Corp.). St. John (1970) has suggested that the peak intensity measurement might not be the most reliable means of determining ATP concentration.

The integral mode of counting has been used with a number of different light detecting instruments which include specially constructed photomultiplier assemblies (Holm-Hansen and Booth 1966), fluormeters (Beutler and Baluda 1964), a commercially available photometer (JRB Co., San Diego, Calif.), and the liquid scintillation counter (Strange et al.

1963; Lin and Cohen 1968; Schram 1970; Schram and Roosens 1972; Rudd and Hamilton 1973).

In our work the emitted light from the firefly reaction was measured on a manual liquid scintillation counter (LSC) (Nuclear Chicago model 4534). For ATP analysis, the scintillation counter was operated in a noncoincident mode using one photomultiplier tube (Schram 1970), and with a maximum window opening of 0.5–10.0 V. For analysis 1 ml of either the sample extract or the ATP standard was added to a chromic acid washed LSC vial, followed by addition of 0.5 ml of 0.025 M Tris buffer (pH 7.75). Next, the vial was closed and 0.5 ml of the enzyme preparation injected into the vial through a small hole drilled in the cap. The vial was immediately inserted into the LSC and exactly 1 min after enzyme injection, a 0.1 min count was taken. This count is an integral measurement of the area under the luminescent decay curve between 60 and 66 s after the start of the reaction. For each enzyme preparation, a standard curve was made by plotting the logarithm of the integral count rate versus the logarithm of the appropriate ATP concentration. Standard solutions containing 1 mg/liter disodium crystalline ATP (Sigma Chemical Company, A 3127) in 0.025 M Tris buffer, were prepared and stored frozen in 10 ml aliquots. When needed, the concentrated solution was diluted in Tris or the acid extracting menstrum to cover a range of 0.3–50 ng/ml ATP.

For some experiments the luminescent decay of the ATP-enzyme reaction mixture was followed by interfacing the LSC with a multichannel analyzer (Nuclear Data model 110) as described by Quammen et al. (1973). The ND-110 was operated in a multi-scaling mode so that each of the 128 channels was opened for data acquisition sequentially for a predetermined counting period over a range of one millisecond to 10 s/channel. A flow cell was used in place of LSC vials to permit mixing of reactants in the LSC detector.

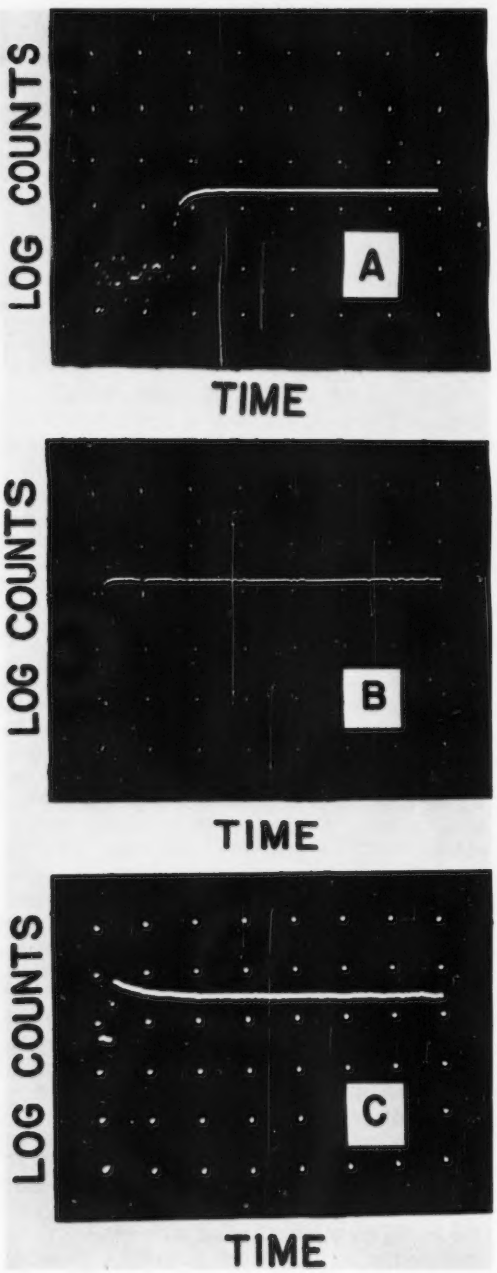
Results and Discussion

Many researchers have used crude, commercially available enzyme extracts reconstituted in 5 ml of distilled water as specified by one manufacturer (Sigma Chemical Company). In our enzyme preparation, however, each vial of lantern extract was reconstituted with magnesium and arsenate salt solutions to yield 25 ml of preparation. Reconstitution of the enzyme to 25 ml permits a greater number of assays to be made and dilutes the endogenous ATP in the enzyme (Schram 1970), thereby reducing the background count rate. The pertinent features of the bioluminescent decay curve using our enzyme preparation are shown in Fig. 1. The rise period, or time required for the light output to reach 60% of the peak intensity after mixing the reactants, was between 100–200 ms (Fig. 1A), and peak intensity count rate was reached within 1 s (Fig. 1B). The subsequent exponential decay period began after 24 s and lasted for up to 20 min (Fig. 1C).

The slopes of the decay curve 60 s after injection of the enzyme were parallel although they were vertically displaced as a function of their ATP content. By measuring the count rate, or more precisely the area under some portion of the decay curve over a fixed time interval, one can construct a standard curve based on an integral measurement. The time interval over which the ATP counts are collected is not critical and we used 0.1 min, the shortest preset time on our LSC. Standard curves were constructed for each enzyme preparation.

In our procedure, we found that the luminescent properties of some LSC vial caps and light exciting the photomultiplier tube as the sample was inserted into the detector greatly increased the apparent count rate and shape of the early portion of the luminescent curve (Quammen et al. 1973). These interferences decay to background levels within 50 s; thus, a 60-s delay period between injecting the enzyme and making the actual ATP measurement was incorporated into the assay procedure.

The lower limit of ATP detectability is influenced by the removal of endogenous ATP from the enzyme (Rasmussen and Nielsen 1968), photomultiplier tube noise, and in the case of an LSC, the use of a non-coincident counting mode (Schram 1970). For a given light detecting instrument the only practical means of altering the lower limit of detectability is to remove endogenous ATP. Gel filtration has been used to remove ATP from enzyme preparations (Rasmussen and Nielsen 1968; Schram and Roosens 1972) but such a purification procedure also removes luciferin which must be replaced prior to enzyme use (Schram and Roosens 1972). Lyman and DeVincenzo (1967) used apyrase to purify their enzyme preparation and reportedly detected ATP concentrations in the picogram levels. Aging the enzyme preparation, i.e. permitting the preparation to stand for some predetermined time before use, also reduces the background count rate. We found aging to be the most convenient method for general use. The background count rate of the enzyme decreased to a minimum level approximately 6 h after reconstitution (Fig. 2). Further aging at room temperature resulted in an increased count rate most probably a result of bacterial growth. Storing the enzyme in an ice bath maintained a lower background level for a



longer period of time. For our particular LSC the lower limit of ATP detection was 0.3 ng.

The maximum statistical variation of the extraction procedure and assay of ATP from environmental samples and bacterial suspensions was ±10% at the 95% confidence interval for samples containing 12 ng/ml of ATP. For a given operator, between-sample variation generally was ±5%.

Extraction Procedures I: Boiling Tris Buffer

The use of boiling Tris buffer for ATP extraction from water or other homogeneous aqueous samples is well documented (Beutler and Baluda 1964; Holm-Hansen and Booth 1966; Holm-Hansen 1969, 1970, 1973a,b; Patterson et al. 1971; Holm-Hansen and Paerl 1972; Quammen et al. 1973).

In our laboratory, attempts to use boiling Tris to extract ATP from beach sands and sediments yielded extremely variable results. The variability may be due to effects of thermal gradients established in a fluid–solid mixture. When solids are submersed in liquids, a transition or boundary layer of fluid is established around the solid that does not have the thermal characteristics of the bulk fluid. This boundary zone may be 0.025–0.25 mm thick. Heat transfer in the bulk fluid is by convection, but within the boundary layer adjacent to the solid heat transfer is by conduction and occurs at a very slow rate (Knudson and Katz 1954). When the fluid is heated, a sharp thermal gradient is established across the boundary layer. While the temperature of the bulk fluid may be near 100 C, at the solid surface it is considerably less. Bacteria adhering to the solid fall within this thermal gradient across the boundary layer, and thus the temperatures to which they are exposed may not be high enough to efficiently extract ATP and prevent its hydrolysis. Patterson et al. (1970) indicated that at

FIG. 1. Reaction kinetics of luciferin–luciferase enzyme preparation. A) Initial rise and slow approach to peak intensity after enzyme injection into 100 ng of ATP. Dwell period was 10 ms per channel; B) Maximum intensity and early decay of peak. Dwell period was 100 ms per channel and ATP concentration was 5ng; C) Initial peak and long lasting exponential decay period. Dwell period was 1 s per channel and ATP concentration was 1 ng. Elapsed time between reference markers in the horizontal direction is 160 ms, 1.6 s, and 16 s for figures A, B, and C, respectively. Reference markers in the vertical direction are the logarithm of counts per channel from $1–10^5$ for all figures.

TABLE 1. Recovery of ATP from *Escherichia coli* suspensions and *Escherichia coli* coated glass beads.

Sample description	No. cells added[a]	ATP added (ng)	ATP extracted (Tris)[b]		ATP extracted (H_2SO_4)[c]	
			ATP (ng)	% Recovery	ATP (ng)	% Recovery
Aqueous bacterial suspension	2×10^8	280	260	92.9	258	92.1
Aqueous bacterial suspension	2×10^8	280	270	96.4	239	85.3
Bacteria coated beads (2 g total)	10^8	140	9	6.4	122.2	87.3
Bacterial coated beads (2 g total)	10^8	140	–	–	131	96.3
Bacteria coated beads (2 g) and uncoated beads (2 g)	10^8	140	5	3.6	114.5	81.8
Bacteria coated beads (2 g) and uncoated beads (4 g)	10^8	140	4.5	3.2	127.5	90.9

[a]Theoretical ATP addition based on intracellular ATP measurement for *E. coli* of 1.4×10^{-15} g per cell.
[b]ATP values assayed using Tris standard curve.
[c]ATP values assayed using H_2SO_4 standard curve.

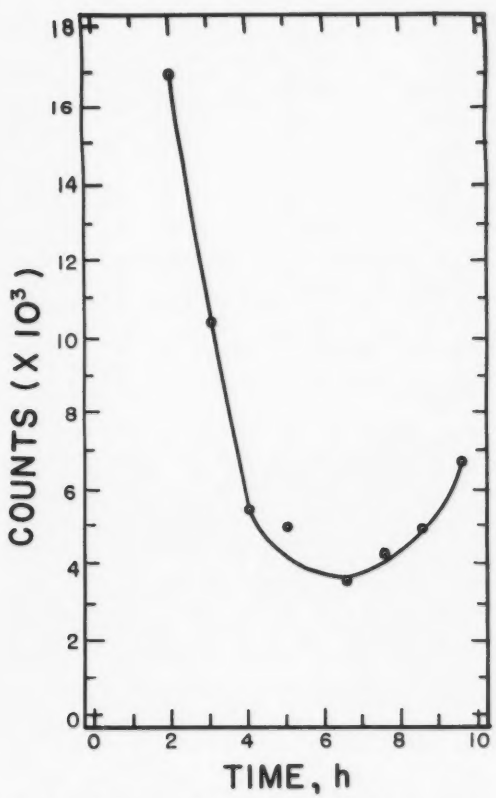


FIG. 2. Temporal changes in background count rate for the luciferin–luciferase enzyme preparation.

temperatures below 90 C the efficiency of ATP extraction with boiling Tris is greatly reduced.

EXTRACTION PROCEDURES II: H_2SO_4-EDTA

A comparison of ATP extraction procedures for soil and sediments has been independently compiled by Lee et al. (1971) and Ausmus (1973). Their results indicated that a cold acid procedure offered the greatest promise for extracting ATP from sediments. The problem of thermal gradients found in hot extraction procedures is avoided as well as the problem of adsorption of ATP by sediment components such as iron and aluminum oxides (Lee et al. 1971). Lee et al. (1971) compared sulfuric acid and a mixture of sulfuric acid and dimethyl sulfoxide (DMSO) and obtained comparable results with either extraction fluid. We found that the DMSO–sulfuric acid extract will not freeze at temperatures as low as –20 C, and that the ATP decayed with time. For this reason all work was done using sulfuric acid alone as the extracting medium for sediment samples.

A comparative experiment was performed to determine the relative efficiencies of boiling Tris and sulfuric acid in extracting ATP from bacterial cells coated on glass beads. The weight of coated beads was kept constant, but uncoated beads were added to determine if the quantity of solid substrate affected either extraction process. The results of this experiment are shown in Table 1. When 10^8 bacterial cells were coated on 2 g of glass beads, Tris extracted only 6.4% of the bacterial ATP in comparison to recoveries of

87 and 94% for the sulfuric acid. Increasing the quantity of glass beads, but maintaining the same bacterial density, further decreased ATP recovery by Tris extraction, but had relatively little effect in the sulfuric acid procedure. The data in Table 1 indicate that the sulfuric acid extraction procedure effectively removed bacterial ATP in the presence of a solid substrate, and in all cases the recovery was greater than 81%, regardless of the amount of solid present.

Attempts at using boiling Tris for extracting ATP from sediments in marine or estuarine environments have been reported (Ernst 1970; Ferguson and Murdoch 1973). In these works an internal ATP standard consisting of either a solution of an ATP salt or lyophilized bacteria was added to the sample to estimate extraction efficiency. In light of the previous discussion such additions will measure only ATP adsorption and ionic interference; they will not measure extraction efficiency of ATP from viable bacterial cells attached to sediment particles and within the fluid boundary layer. A more suitable standard would be bacterial cells coated on glass beads and added to the sample prior to extraction.

Aledort et al. (1966) indicated a marked drop in the light emission in the luciferin–luciferase reaction resulting from the addition of a small concentration of cations. They reported that a Ca^{++} concentration of 97 mM could completely inhibit the luminescent reaction, and that Na^{+} also greatly decreased light emission. These considerations are particularly critical when using an acid extraction procedure on marine sediments containing calcium carbonate.

A series of experiments was performed to determine the relative effects of selected anions and cations commonly found in sea water, using the sodium or chloride salts. Each cation or anion concentration was assayed in triplicate and the results were averaged. Maximum variation between any of the triplicates was never more than 5% of their average. The results of these experiments indicate: (1) At the highest concentrations tested (100 mM), all cations or anions tested inhibited light emission by 60 and 100% (Fig. 3, 4; Table 2). (2) Equimolar concentrations of anions inhibit in the order of $CO_3 = >PO_4 \equiv >SO_4 = >Cl-$ (Fig. 4). (3) Equimolar concentrations of cations inhibit in the order $Ca^{++} > K^{+} \geqslant Na^{+} > Mg^{++}$ (Fig. 3). (4) Light emission decreased linearly in relation to the logarithm of the cation or anion concentration with the exception of magnesium (Fig. 3, 4). (5) For a given molar concentration of the cation, the inhibition is more pronounced at lower ATP values (Table 2). For example, a Ca concentration of 50 mM permits only 5.9% light emission with an ATP concentration of 0.7 ng compared to 21% light emission with 70 ng of ATP (Table 2). Ionic inhibition in the luciferin–luciferase reaction, therefore, is a function of the nature of the anion or cation, its concentration, and the concentration of ATP in the sample. The net effect of these variables can best be determined using an internal ATP standard for each environmental sample.

Ionic inhibition by sulfate ions suggests that acid extracted samples will have a lower count rate than equivalent Tris extracts. A comparison of a series of ATP standards prepared in Tris buffer and sulfuric acid showed that for any given ATP concentration the count rate in sulfuric acid menstruum is less than that obtained with Tris

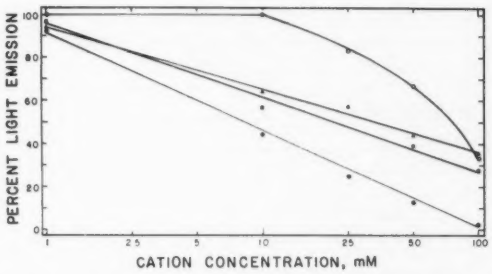


FIG. 3. Decrease in light emission in the luciferin–luciferase reaction as a function of cation concentration. All cations were added as chloride salts and the ATP concentration was 7 ng/ml. Symbols: open circle, Mg^{++}; solid circle, Ca^{++}; triangle, Na^{+}; square, K^{+}.

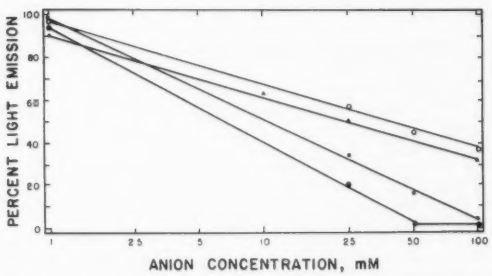


FIG. 4. Decrease in light emission in the luciferin–luciferase reactions as a function of anion concentration. All anions were added as sodium salts and the ATP concentration was 7 ng/ml. Symbols: open circle, Cl^{-}; solid circle, $CO_3^{=}$ triangle, $SO_4^{=}$; square, $PO_4^{\equiv}$.

TABLE 2. The interference of calcium and magnesium ions on light emission in the luciferin–luciferase reaction for ATP solutions of varying concentration.

Cation added[a]	Cation concentration (m*M*)	ATP concentration (ng)	% Light emission[b]
Ca	100	0.7	0
Ca	100	7	2.7
Ca	100	70	5.4
Ca	50	0.7	5.9
Ca	50	7	12.4
Ca	50	70	21
Ca	25	0.7	18.6
Ca	25	7	22.4
Ca	25	70	35.6
Ca	10	0.7	38
Ca	10	7	48
Ca	10	70	80.5
Ca	1	0.7	93
Ca	1	7	96
Ca	1	70	99.5
Mg	100	0.7	33
Mg	100	7	34
Mg	100	70	44.5
Mg	50	0.7	62
Mg	50	7	68
Mg	50	70	71.5
Mg	25	0.7	70.5
Mg	25	7	82
Mg	25	70	88
Mg	10	0.7	93
Mg	10	7	100
Mg	10	70	96
Mg	1	0.7	96
Mg	1	7	100
Mg	1	70	102

[a]Cations were added as Cl^- salts.

[b]ATP count rate with no exogenous cations was taken as 100% light emission.

buffer (Fig. 5). The difference between the two menstruums is not constant, however, and decreases with increasing ATP concentration. This observation is also consistent with the cation effects reported in Table 2. Because of the difference in count rate obtained with H_2SO_4 or Tris extractants, it is essential that ATP in a sulfuric acid menstruum be assayed and compared to a standard curve made up with sulfuric acid. Lee et al. (1971) used a Tris standard curve in evaluating the efficiency of the various extracting fluids they tested, leading to an underestimate of the ATP content.

Lee et al. (1971) suggested using a cation exchange resin to mitigate the overall ionic inhibition. The exchange of sodium for various metal ions on an equal charge basis would not

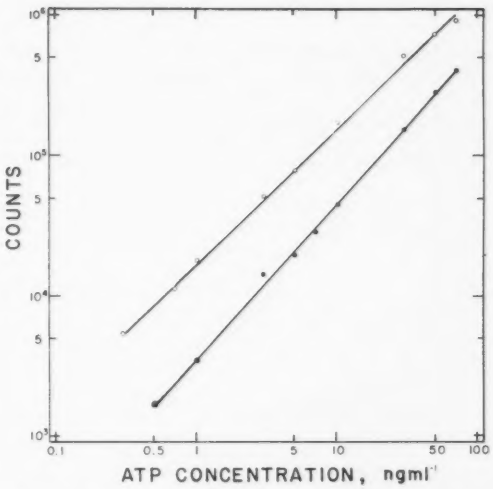


FIG. 5. Counts obtained for ATP standards in Tris and sulfuric acid menstruums, using the counting procedure outlined in Extraction Procedures II. Symbols: open circle, Tris buffer; closed circle, sulfuric acid.

appear to significantly reduce interference as one cation is exchanged for another that will also interfere.

Complex formation was investigated as a means of reducing the concentration of free metal ions in solution using EDTA, which has a high specificity for divalent cations, especially calcium (Perrin 1964). The addition of EDTA may be necessary to promote light emission in samples of low ATP content, where ionic inhibition is extensive and light emission otherwise totally inhibited. The effect of added EDTA to a sample containing 10 ng of ATP in H_2SO_4, is to first increase the count rate to a maximum with approximately 8 mg EDTA (Fig. 6). At greater concentrations of EDTA the count rate decreases and at 20 mg it is equivalent to the count rate obtained without EDTA. Further additions drastically suppress light output to levels below the enzyme background count rate. Presumably the decrease in light emission is a result of complexing magnesium ions which are essential for the luciferin–luciferase reaction.

A second experiment was performed in which EDTA was added to a solution containing 10 ng ATP and 10 mM Ca^{++}. The results of this experiment are shown in Fig. 7, indicating again an initial enhancement followed by suppression of light emission. The principal difference between Fig. 6 and 7 is that in the presence of interfering

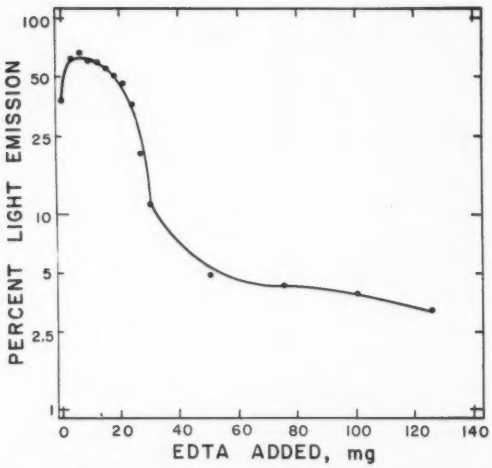


FIG. 6. The effect of EDTA additions on light emission in the luciferin–luciferase reaction. The ATP concentration was 10 ng/ml and the enzyme background count was 4.4%.

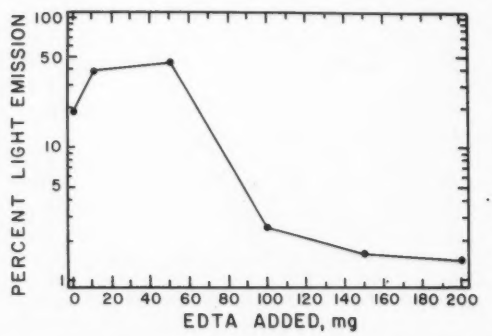


FIG. 7. The effect of EDTA additions on light emission in the luciferin–luciferase reaction in the presence of 10 mM Ca^{++}. The ATP concentration was 10 ng/ml and the enzyme background count was 2.5%.

cation, greater quantities of EDTA can be added before the light reaction is suppressed. The exact amount of EDTA to be added is thus dependent on the concentration of ionic species present in the sample. Based on the results of Fig. 6 and 7, a solution of 0.048 M EDTA was prepared and 1 ml of this solution added to each extracted sample. This corresponds to an EDTA addition of 18 mg/sample.

The hydrolysis of ATP in sulfuric acid–DMSO extracted samples raised the question whether extracted ATP would decay in an acid solution. An experiment was performed in which 3 and 10 ng/ml ATP standards were prepared in the H_2SO_4 extracting menstruum and distributed in a series of test tubes. At the desired time one tube of each ATP concentration was neutralized with NaOH and assayed. The results of this experiment indicated no loss of ATP occurred over 65 min as a result of hydrolysis in an acid solution. Once neutralized and frozen, acid extracted samples were stable for periods up to 1½ mo, the longest storage period we tested.

Conclusions

1) Reconstitution of lyophilized enzyme with arsenate buffer and magnesium sulfate to a total of 25 ml yielded a preparation that gave consistent results, reduced background count rate, and permitted a large number of samples to be assayed.

2) Cations and anions interfere with light emission in the luciferin–luciferase assay. In the presence of high concentrations of ions, light emission can be totally inhibited. The interference is dependent upon the specific ions encountered and the ATP concentration in the sample.

3) Boiling Tris buffer is a suitable menstruum for extracting samples concentrated on membrane filters but is unsatisfactory when applied to sediments. The concentration of detrital material on membrane filters along with the microbes, may necessitate the use of an internal ATP standard to determine losses resulting from ATP adsorption.

4) A sulfuric acid menstruum offered greater, more uniform ATP recovery from sediments than did the boiling Tris buffer. The addition of EDTA lessens the ionic effects and promotes light emission. The magnitude of all sources of interference can be determined with an internal ATP standard.

Acknowledgments

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Toxaphene Effects on Growth and Development of Brook Trout (*Salvelinus fontinalis*)

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MEHRLE, P. M., AND F. L. MAYER JR. 1975. Toxaphene effects on growth and development of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 32: 609–613.

Twenty-two days before hatching, eyed eggs of brook trout (*Salvelinus fontinalis*) were placed in a flow-through system and continuously exposed to toxaphene (0, 39, 68, 139, 288, and 502 ng/liter) until they hatched and the resulting fry were exposed for a further 90 days. Hatchability was not affected by toxaphene, but all of the fry exposed to 502 and 288 ng/liter of toxaphene died by 30 and 60 days after hatch, respectively. Growth of fry was significantly depressed at the 139 and 288 ng/liter concentrations 30 days after hatch and at all concentrations after 60 and 90 days of toxaphene exposure.

Whole body collagen of fry, as estimated by hydroxyproline, was significantly decreased in the four higher concentrations of toxaphene within 7–15 days after hatch. Backbone collagen was significantly decreased ($P < 0.05$) in 30-, 60-, and 90-day-old fry exposed to toxaphene, whereas calcium and phosphorus concentrations in the backbone were increased ($P < 0.05$). The mineral: collagen ratio was significantly increased by toxaphene. The whole body collagen of sac-fry was a good predictor of later growth and development of brook trout.

MEHRLE, P. M., AND F. L. MAYER JR. 1975. Toxaphene effects on growth and development of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 32: 609–613.

Des œufs oculés d'omble de fontaine (*Salvelinus fontinalis*) furent placés, 22 jours avant éclosion, dans un appareil traversé par un débit d'eau et exposés de façon continue à du toxaphène (0, 39, 68, 139, 288 et 502 ng/litre) jusqu'à éclosion. Les alevins qui en résultèrent furent exposés pendant une période additionnelle de 90 jours. Le succès de l'éclosion n'est pas affecté par le toxaphène. Par contre, tous les alevins exposés à 502 et 288 ng/litre de toxaphène meurent en dedans de 30 et 60 jours, respectivement, après éclosion. La croissance des alevins est nettement ralentie, aux concentrations de 139 et 288 ng/litre, 30 jours après éclosion et, à toutes les concentrations, après 60 et 90 jours d'exposition au toxaphène.

Le collagène du corps entier des alevins, estimé par hydroxyproline, diminue de façon significative aux quatre concentrations de toxaphène les plus fortes en dedans de 7–15 jours après éclosion. Le collagène de la colonne vertébrale est nettement plus bas ($P < 0.05$) chez les alevins de 30, 60 et 90 jours exposés au toxaphène, alors que les concentrations de calcium et de phosphore de la colonne vertébrale sont plus élevées ($P < 0.05$). Le rapport minéraux: collagène augmente notablement sous l'influence du toxaphène. Le collagène du corps entier de l'alevin vésiculé donne une bonne indication de ce que seront plus tard la croissance et le développement de l'omble de fontaine.

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ALTHOUGH the use of many organochlorine insecticides has been reduced in recent years, about 30–40 million lb of toxaphene are still applied annually to crops and livestock (Hercules Inc. 1970). As the use of DDT was restricted in 1969, toxaphene is often used to replace it, alone or in combination with other insecticides (Courtenay and Roberts 1973). Acute effects of toxaphene on aquatic organisms were reported by several investigators (Doudoroff et al. 1953; Katz 1961) and reviewed by Mayer et al. (1975), and the chronic, sublethal effects of the compound on growth and reproduction of brook trout (*Salvelinus fontinalis*) were investigated by Mayer et al. (1975).

The present investigation was conducted simultaneously with the foregoing study of chronic effects, but we focus here on selected biochemical

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processes basic to growth and development of young brook trout.

As proper skeletal development is essential to growth, we concentrated our efforts on the synthesis of collagen, the fibrous matrix of skeletal structures. Collagen, as estimated by synthesis of hydroxyproline, was used as a representative "differentiated" protein for study of development as reported in amphibian embryological investigations by Rollins and Flickner (1972) and by Green et al. (1968). They reported that collagen synthesis is repressed during the first cleavage stages of frog embryos (*Xenopus laevis*) but begins during gastrulation and increases 500-fold through neurulation, hatching, and posthatching stages. We correlated the effects of toxaphene on growth and development of brook trout with effects on collagen content as measured by hydroxyproline metabolism to 1) support the conventional length and weight measurements for growth and 2) to ascertain if a biochemical method for evaluating growth and development could be established that would be useful in predicting the no-effect concentration of toxaphene after a short exposure, before changes in weight or length become apparent.

Materials and Methods

Eggs of brook trout were continuously exposed to toxaphene in a proportional diluter system modeled after that described by Mount and Brungs (1967). The diluter with a modification of McAllister et al. (1972), delivered five concentrations of toxaphene and a control, with a dilution factor of 0.5 between the concentrations. Acetone, the carrier solvent for toxaphene, did not exceed 0.28 mg/liter in tanks treated with toxaphene. Toxaphene concentrations in water were measured weekly by gas–liquid chromatography (Stalling and Huckins 1975). Average measured concentrations were 0, 39, 68, 139, 288, and 502 ng/liter; analytical sensitivity was 10 ng/liter. Flow-splitting chambers (Benoit and Puglisi 1973) were used to thoroughly mix and divide each toxaphene concentration for delivery to duplicate stainless steel tanks 51 cm × 138 mm long with a water depth of 30 cm. An experimental use sample of toxaphene (X-16189-49) was furnished by Hercules Inc. and was used throughout the study. The water temperature was maintained at 9 C, and artificial daylight was provided by the method of Drummond and Dawson (1970). The water temperature and photoperiod were those recommended by the U.S. Environmental Protection Agency (1972) for brook trout tests. Water characteristics were pH 7.2, alkalinity 237 mg/liter, and hardness 272 mg/liter.

One hundred eyed brook trout eggs, placed in each of six incubator cups per duplicate tanks, were exposed to toxaphene for 22 days before the median hatch date and the resulting fry were exposed for an additional 90 days. When hatching was complete, four groups of 25 fry were randomly selected from among the six incubation cups and placed in growth chambers within the tank. The growth chambers were 38 × 15 × 14 cm deep; water depth was 10 cm. The remaining fry were held in the tank for toxaphene uptake and biochemical determinations (hydroxyproline, calcium, and phosphorus). Fry were fed the commercial diet, Ewos, ad lib. Total lengths of fry were determined by the photographic method of McKim and Benoit (1971) at hatching and at 30, 60, and 90 days after hatch. Weight of the fry was determined at 90 days.

Eight brook trout fry from each concentration were sampled at 7, 15, 30, 60, and 90 days after hatching for biochemical analyses. The fry from the 7- and 15-day samples were blotted to remove excess water, weighed, homogenized in 2 ml of 10% trichloroacetic acid, and centrifuged at 1500 rpm for 5 min. The supernatant was discarded; the protein precipitate was washed twice with distilled water and hydrolyzed at 110 C in 3 ml of 6 N HCl for 16 h. The hydrolysate was diluted to 25 ml with distilled water and hydroxyproline (HyP) determined in a 2-ml aliquot according to the method of Woessner (1961). Detection of HyP in the protein hydrolysate was used as the indicator for collagen, as HyP is restricted to the proteins collagen and elastin, and only minor amounts are contributed by elastin (Rollins and Flickner 1972). Fry from the 30-, 60-, and 90-day samples were blotted and placed on dry ice. After the fry were frozen, the backbone of each fish was removed, dried at 110 C in a forced-air oven for 2 h, and then weighed. The dried bone was subjected to hydrolysis at 110 C in 3 ml 6 N HCl for 16 h. The hydrolysate was diluted to 10 ml with distilled water. HyP in the hydrolysate, was determined by the method of Woessner (1961), phosphorus by a modification of the spectrophotometric method of Fiske and Subbarow (1925), and calcium by atomic absorption spectrophotometry. The precision of each of the methods varied by less than 3%, and the recovery from spiked samples was 95–99%.

Samples of 30, 25, 20, 15, and 10 brook trout fry were taken from each duplicate tank after 7, 15, 30, 60, and 90 days of exposure, respectively, for determination of toxaphene residues. We pooled the fry from each duplicate for residue analysis, using the procedures of Stalling and Huckins (1975). A randomized block design was utilized and the data were analyzed by analysis of variance to determine if significant differences existed. A multiple means comparison test (least significant difference) was used to compare differences among treatments (Snedecor 1965).

Results and Discussion

Toxaphene had no effect on hatchability of brook trout eggs. This suggests that during the

22-day period prior to hatching the mechanisms responsible for hatching were not affected by toxaphene. The amount of toxaphene accumulated by the eggs during this period of exposure was not determined. Further research can elucidate the residue dynamics in eggs, as well as the sensitivity of eggs to toxaphene during earlier developmental stages.

After hatching the fry accumulated toxaphene rapidly. In 15 days, the fry had concentrated toxaphene by 67,000–76,000 times the concentration they were exposed to in water (Table 1). Uptake at all toxaphene concentrations reached a peak after 15 days of exposure, declined through 60 days, and then tended to increase again between 60 and 90 days. The possible reason for the greater uptake of toxaphene during the first 15 days may be due to anatomical changes during this developmental phase. As gills are generally not functional until the yolk sac is absorbed, most respiration during this period occurs through a vascular network enveloping the yolk (Brown 1957) and toxaphene may be readily absorbed and stored in the yolk in glyceride fat droplets. These lipids are utilized toward the end of the yolk-sac phase of development (Brown 1957). Shortly after the yolk sac is absorbed and just before the fry begin to feed, fat content is probably lowest and therefore the equilibrium between toxaphene in the water and that in the fish is low. As the fry begin to feed and lipid content increases, the accumulation of toxaphene again increases.

Toxaphene had no effect on weight gain within 7 or 15 days after hatching. However, collagen content, as estimated by direct measurement of HyP in the protein fraction, was significantly ($P<0.05$) decreased after both 7 and 15 days in all fish except those in the 39 ng/liter concentration (Table 2). The decrease in whole body collagen implies that the precursor molecules of the organic matrix of skin, bone, and other collagen-containing tissues were significantly reduced.

TABLE 1. Whole-body residues (μg/g) of toxaphene in brook trout *(Salvelinus fontinalis)* fry[a] continuously exposed to toxaphene.

Water concn (ng/liter)	Toxaphene residues after: 7 (days)	15 (days)	60 (days)	90 (days)
0	–[b]	–	–	–
39	0.2	2.6	0.4	0.6
68	1.0	3.7	0.9	1.4
139	2.2	8.3	1.8	2.6
288	4.5	18	–	–
502	9.2	38	–	–

[a]Mean of two composite samples.
[b]No detectable residue.

All of the fry exposed to 502 ng/liter of toxaphene died within 30 days and those exposed to 288 ng/liter within 60 days. Growth of the fry, as measured by length, declined ($P<0.05$) within 30 days after hatching in the 139 and 288 ng/liter concentrations (Table 3). After 60 days, the growth of fry as measured by length was lower ($P<0.05$) at all toxaphene concentrations.

All toxaphene concentrations significantly altered ($P<0.05$) the composition of brook trout backbones after 30, 60, and 90 days of exposure (Table 4). Effects were more pronounced after 90 days of exposure than after 30 and 60 days. Collagen content in backbone, as measured by HyP, was the only characteristic that was significantly decreased after 30 days of exposure. The results were similar to those from the 7 and 15 day whole-body samplings, i.e. collagen content was decreased by toxaphene. The decrease in bone collagen suggests that the effect of toxaphene on collagen synthesis during the first few days of life significantly affected the development of bone tissue.

In addition to the significant reduction in collagen concentration, the phosphorus concentration in the bone from the 139 ng/liter treatment group was significantly decreased ($P<0.05$) after 60 days of exposure. The calcium concentration was unchanged in all toxaphene concentrations. We do not understand the significance of the decrease in phosphorus concentration at 60 days and the later increase at 90 days. However, the decline in collagen content between the 30- and 60-day sampling periods appears highly significant and may be due to the increase in mineralization which occurred as the backbone matured. The process of mineralization appears to be most extensive between the 60-and 90-day periods, as judged by the increase in phosphorus and calcium concentrations.

After 90 days of exposure, toxaphene had an even more dramatic effect on bone composition (Table 4). In addition to the significant decrease in collagen content, both the calcium and phosphorus concentrations were increased ($P<0.05$) by about 100% in fish exposed to the 68 and 139 ng/liter concentrations and there was a trend towards an increase in Ca and P in the 39 ng/liter treatment group. The ratio of minerals:organic content (Ca+P/collagen) of the backbone in the control group was 1.07, whereas the ratio was

TABLE 2. Collagen synthesis as estimated by hydroxyproline content in brook trout sac fry exposed to toxaphene. Values represent the mean of eight determinations expressed as micrograms hydroxyproline per gram fry.

Time after hatching (days)	Toxaphene concn (ng/liter)					
	0	39	68	139	288	502
7	649	607	463[a]	498[a]	459[a]	481[a]
15	783	765	683[a]	588[a]	650[a]	532[a]

[a]Significantly different from controls ($LSD_{0.05}$).

TABLE 3. Growth of brook trout fry continuously exposed to toxaphene: mean length (millimeters) after successive periods of exposure and weight (grams) after 90 days (standard deviations in parentheses).

Toxaphene concn (ng/liter)	Fry length (mm)				Weight (g) at 90 days
	0 day	30 days	60 days	90 days	
0	16.5 (0.8)	23.4 (1.2)	34.3 (4.4)	42.0 (9.2)	0.81 (0.60)
39	16.8 (1.1)	23.3 (2.2)	31.4[a] (3.3)	35.8[a] (6.2)	0.44[a] (0.30)
68	16.8 (1.1)	23.6 (2.0)	31.3[a] (3.4)	38.3[a] (8.2)	0.59[a] 0.44
139	16.8 (1.2)	22.0[a] (2.1)	30.8[a] (4.3)	35.1[a] (7.7)	0.43[a] (0.37)
288	16.3 (1.0)	19.4[a] (1.6)	–[b]	–[b]	–[b]
502	16.4 (1.0)	–[b]	–[b]	–[b]	–[b]

[a]Significantly different from controls ($LSD_{0.05}$).
[b]All fish dead.

1.89, 2.52, and 2.50 in the 39, 68, and 139 ng/liter treatment groups, respectively. The increase in the ratio of minerals to collagen content suggests that the fragility of the backbones increased.

The data presented here strongly suggest that toxaphene inhibited collagen synthesis. This inhibition depressed both whole-body collagen content of sac-fry and backbone collagen in fry and, concomitantly, increased mineralization of the backbones. However, whether or not toxaphene directly altered mineral metabolism is not known. We suggest that the primary effect of toxaphene is on collagen metabolism, and that the effect on mineral metabolism is secondary. We have induced a similar condition with toxaphene in fathead minnows (*Pimephales promelas*) (Mehrle and Mayer 1975). The backbones of fathead minnows exposed to toxaphene were fragile and easily broken when subjected to a sublethal electric shock. The results from the present study imply that toxaphene can induce a similar condition in brook trout.

At present, chronic studies are used to determine no-effect concentrations of toxicants. However, the results of the present investigation suggest that it may be feasible to predict no-effect concentrations in salmonids by using a biochemical measurement that is related to growth and development, such as whole-body or backbone collagen. For example, 7 days after hatching the no-effect concentration on growth and development by determination of whole-fry collagen was estimated to be between 39 and 68 ng/liter, and on the basis of backbone collagen the no-effect concentration was less than 39 ng/liter at 30 days after hatching. However, length and weight were not changed in the fish exposed to all concentrations of toxaphene until after 30 days of exposure. Thus, collagen measurements appear to be reliable biochemical predictors or sensitive indicators of altered growth and development. Use of such predictors in toxicological research could reduce exposure time in estimating the effects of toxicants on growth.

TABLE 4. Backbone composition of brook trout fry exposed to toxaphene. Mean values expressed on basis of dried weight of backbone.

Days after hatching, and backbone constituent[a]	Toxaphene concn (ng/liter)				
	0	39	68	139	288
30 days					
Collagen[b]					
(mg HyP/g)	51.34	34.66[c]	26.16[c]	19.76[c]	24.99[c]
Phosphorus (%)	5.73	7.30	9.89	8.80	10.90
Calcium (%)	1.23	0.98	1.33	1.05	1.43
60 days					
Collagen					
(mg HyP/g)	20.10	13.86[c]	14.02[c]	10.98[c]	–[d]
Phosphorus (%)	12.05	10.81	9.89	7.71[c]	–
Calcium (%)	2.64	2.85	2.54	1.31	–
90 days					
Collagen					
(mg HyP/g)	19.48	16.42[c]	16.22[c]	16.42[c]	–
Phosphorus (%)	10.60	15.61	20.25[c]	19.87[c]	–
Calcium (%	10.20	15.34	20.61[c]	21.14[c]	–

[a] n = 6–8.
[b] Collagen estimated by determination of hydroxyproline.
[c] Significantly different from controls ($LSD_{0.05}$).
[d] All fish exposed to 288 ng/liter toxaphene died within 60 days and all those exposed to 502 ng/liter (not shown) died within 30 days.

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Chemical Changes During the Seasonal Cycle of Growth and Decay in Eelgrass (*Zostera marina*) on the Atlantic Coast of Canada

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HARRISON, P. G., AND K. H. MANN. 1975. Chemical changes during the seasonal cycle of growth and decay in eelgrass (*Zostera marina*) on the Atlantic coast of Canada. J. Fish. Res. Board Can. 32: 615–621.

In a submerged bed of eelgrass, newly formed leaves in winter and spring had their maximum levels of total organic matter (90% of dry weight), soluble organic fraction (45%), carbon (42%), and nitrogen (4.8%). These components all decreased as the leaves matured, aged, and died. Soon after death, a leaf had only 70% total organic matter, 28% soluble organic matter, 30% carbon, and 1.5% nitrogen. Intact dead leaves showed little further change in chemical composition. The commonly used crude protein determination (6.25 × nitrogen content) overestimated true protein by up to 180%. The carbon to nitrogen ratio (C:N) was an unreliable index of the nutritional value of the plant. Two growth forms were present, most probably in response to wave action and substrate composition. Daylength, not temperature, most probably controls the seasonal cycle of growth.

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Dans un lit submergé de zostères marines, les feuilles nouvellement formées en hiver et au printemps ont leurs niveaux maximums de matière organique totale (90% du poids sec), fraction organique soluble (45%), carbone (42%) et azote (4.8%). Ces constituants diminuent tous à mesure que les feuilles mûrissent, vieillissent et meurent. Peu après la mort, une feuille ne contient que 70% de matière organique totale, 28% de matière organique soluble, 30% de carbone et 1.5% d'azote. La composition chimique des feuilles mortes intactes ne change que très peu par la suite. La méthode sommaire communément employée pour la détermination des protéines (6.25 × contenu en azote) donne des valeurs dépassant jusqu'à 180% la valeur réelle des protéines. Le rapport carbone : azote (C : N) est un indice peu fiable de la valeur nutritive de la plante. Il existe deux formes quant à la croissance, très probablement en réponse à l'action des vagues et à la composition du substrat. La longueur du jour, non la température, contrôle très probablement le cycle de croissance saisonnier.

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MANY of the animal communities in coastal waters, from benthic infauna to fish, are thought to depend on detritus originating from macrophytes such as seagrasses, marsh grass, and kelp (Mann 1972). Eelgrass (*Zostera marina*) is the most common seagrass in northern Atlantic and Pacific waters where it often dominates the submerged vegetation of shallow, sedimented areas. Eelgrass forms extensive, highly productive meadows inhabited by many species of animals (Kikuchi and Pérès 1973; McRoy and McMillan 1973), but little of the living plant biomass is grazed. Instead, most of the leaves die and begin to decompose before animals consume them (Fenchel 1972).

Extensive investigations early in this century in Denmark indicated the importance of eelgrass to coastal productivity (Ostenfeld 1908; Petersen and Boysen-Jensen 1911; Boysen-Jensen 1914; Petersen 1915, 1918). Despite this early work

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and more recent studies by Burkholder and Doheny (1968), McRoy (1968), Fenchel (1972), and Phillips (1972), additional data on the autecology of eelgrass are needed to fully understand the role of eelgrass detritus. This study documents the seasonal cycle of growth and decay in terms of some major chemical constituents of the plant that are important in trophic relations. Also investigated, because of conflicting reports in the literature, were the timing of the various stages of the cycle and the relation of the growth of the plant to water temperature and other environmental factors.

Site and Methods

The source of eelgrass was an extensive submarine meadow off Troop Island in St. Margaret's Bay on the Atlantic coast of Nova Scotia (Fig. 1). The eelgrass bed covers 1.7 ha and is completely subtidal (Fig. 2). Situated between the mainland and an island, the bed is never subjected to severe wave action but tidal currents are occasionally strong enough to bend the plants at about a 60° angle to the sediment surface. Vegetative plants and loose leaves were collected using SCUBA at intervals of 2–6 wk from September 1972 to September 1973. Plants taken from several spots within the bed were pooled for analysis. On each sampling date the water temperature was recorded, and notes were made on the general condition of the plants.

Plants were transported in water-filled plastic buckets to the laboratory where leaves were removed at the junction of leaf and sheath. The sheath was not used because field observations confirmed that it remained attached to the rhizome at the time of leaf abscission. Thus only one part of the plant, the leaf material that dropped off and entered the detritus system, was analyzed. Leaves were sorted into four classes: 1) Green — no signs of deterioration; 2) Old — with brown or black areas, senescing; 3) Dead, in shallow water — loose on the sediment among the living plants; 4) Dead, in deep water — loose on the sediment in 10–13 m of water where no plants grow, presumably carried there by water movements. The leaves were cleaned of macroscopic epibionts (mainly filamentous red and brown algae, and bryozoans), and rinsed in fresh sea water.

Three methods have been used commonly for estimating the protein content of aquatic macrophytes: 1) the sum of the amino acids joined by peptide bonds, which requires expensive equipment and/or many time-consuming analyses (Boyd 1970); 2) 6.25 × trichloroacetic acid-insoluble nitrogen, which closely approximates true protein (Pirie 1955); and 3) 6.25 × total nitrogen, which gives a quick but crude estimate (Pirie 1955). For protein determination by method (2), 5.0-g portions of clean, fresh leaves were macerated in a Waring blender in 100 ml of a 5% (wt/vol) solution of trichloroacetic acid (TCA) in distilled water. This was made up to 400 ml with 5% TCA, boiled 1 h, filtered through Whatman #1 paper, rinsed with distilled water, and dried at 100 C for 12 h. The TCA-insoluble residue was weighed and expressed as a percent of the original dry weight (determined as below). Total nitrogen of the residue was measured with the CHN Analyzer (see below).

$$\text{\% Protein–nitrogen in dry leaf} = \text{\% Nitrogen in TCA-insoluble residue} \times \text{TCA-insoluble residue as \% of dry leaf weight}$$

For all other analyses, clean leaves were blotted "dry," weighed, dried 12–24 h at 100 C (to constant weight), reweighed, and the dried material was ground in a Wiley mill until it passed through a sieve with 420 μm openings.

Organic content was calculated as loss of dry weight on ashing of triplicate samples for 8–12 h at 520 C. Duplicate samples were also digested in a solution of neutral detergent (Van Soest and Wine 1967; Polisini and Boyd 1972). The undigested residue is called the "cell-wall" fraction. Soluble weight

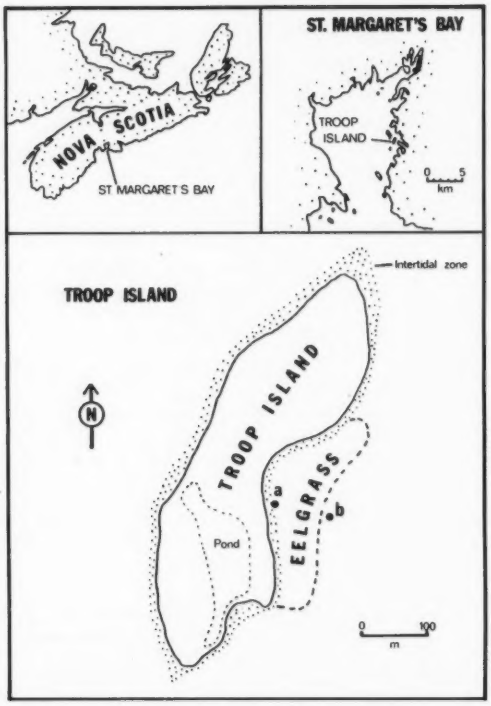

FIG. 1. Troop Island, the eelgrass bed, and the ends of Transect a-b. Insets show the locations of St. Margaret's Bay in Nova Scotia and of the island in the bay.

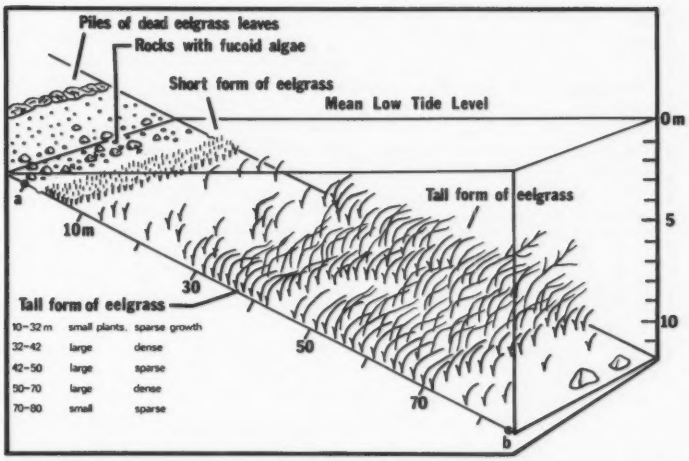

FIG. 2. Distribution with depth of tall and short forms of *Zostera marina* on Transect a-b. Distance along transect and depth are on different scales.

was calculated as the difference between total and residual weight. Cell-wall organic matter (and hence soluble organic matter by difference) was determined from loss of weight on ashing.

Ground leaf material was analyzed for carbon and nitrogen with a Hewlett Packard F & M Scientific Model 185 Carbon–Hydrogen–Nitrogen analyzer calibrated with cyclohexanone-2,4-dinitrophenyl hydrazone (C_6H_{10}:N NH C_6H_3 $(NO_2)_2$).

Results and Discussion

Two growth forms of *Z. marina* were present (Fig. 2) — a) a form with short leaves (up to 18 cm long and 2.5 mm wide) in a dense mat in water less than 1 m deep, and b) a form with longer, wider leaves (up to 50 cm long and 4 mm wide) which grew in deeper water in soft mud. This study concerns the second form because it covered most of the area and constituted most of the biomass. Eelgrass has been reported to exhibit varied growth forms in different localities, but the reasons given by various authors are unsupported by experimental evidence and tend to be conflicting. For example, Burkholder and Doheny (1968) thought light limited growth in turbid waters whereas Ostenfeld (1908) and Philip (1936) attributed good growth to a nutrient-rich muddy substrate. McRoy (1970a) added temperature to the list of environmental factors which may influence growth form; low temperatures, he surmised, prolong the vegetative phase and enable the plant to grow larger. In this study temperature was unimportant; tall and short forms grew in the same water temperatures (Fig. 2). Neither was light the controlling factor, for the short plants grew in shallow water where light was abundant. In contrast to the Troop Island site are more protected inlets where wave action is negligible and where tall plants commonly grow in mud at all depths up to the low tide mark. It is suggested, therefore, that wave action and the coarse nature of the substrate may be restricting upward growth in shallow water at Troop Island, whereas the thicker layer of nutrient-rich mud observed in deep water may be responsible for the lush growth there.

The seasonal growth cycle (Fig. 3, Table 1) does not bear the same relation to temperature as that reported by Setchell (1929). First, most of the vegetative growth occurred at temperatures less than 10 C, Setchell's lower limit for growth. Several other authors have reported only limited growth at such low temperatures (Ostenfeld 1908; Burkholder and Doheny 1968; McRoy 1968). Second, this study, like that of Phillips (1969) in Puget Sound (Washington), showed that even sexual reproduction occurred in waters less than 10 C. Most previous investigators have agreed with Setchell that the bulk of the vegetative growth occurs between 10 and 15 C, and flowering occurs only between 15 and 20 C (Setchell 1929; Tutin 1942; Burkholder and Doheny 1968; McRoy 1969; den Hartog 1970; Rasmussen 1973). If temperature is the controlling factor, then, although in all cases growth increases with rising temperature, and vice versa, plants in Nova

TABLE 1. Indicators of growth seasons in *Zostera marina*.

Season	Stage of plant growth at start	Stage of plant growth at end	Water temp (C) start of season
Spring	initiation of rapid vegetative growth	peak of vegetative growth	3
Summer	flowering	seeds ripen	7
Fall	rapid aging of leaves	most dead leaves abscise	15–20
Winter	dead leaves abundant	spring leaf growth	5

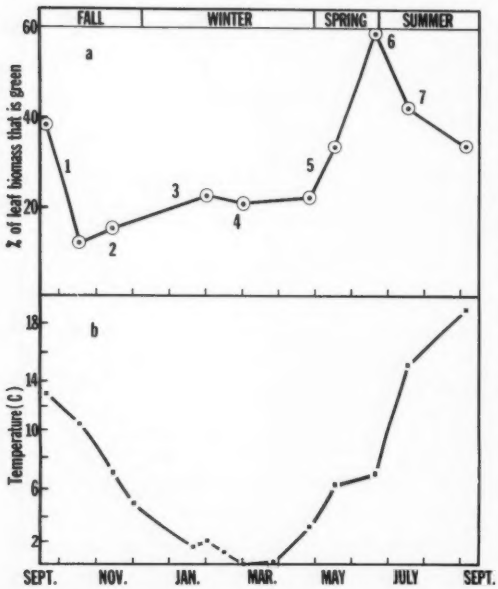

FIG. 3a) Percent of leaf biomass that is green at various times of year. Major events in the cycle of growth and decay are: 1) many leaves senesce and fall off the plant, epiphytic bryozoans common; 2) most of the aging leaves have dropped off, piles of dead leaves accumulate in eelgrass bed; 3) a few short green leaves are present; 4) piles of dead leaves collect around rocks in deeper water; 5) remaining senescent leaves fall off, new leaves begin rapid growth; 6) flowers are abundant; 7) seeds begin to ripen, leaves mature rapidly. b) Water temperature at time of sampling plotted against time of year.

Scotia and Washington are adapted to a very different temperature regime than Setchell proposed. If, however, one considers that in each case referred to, spring growth began as the day-length increased, then light is more likely to be the true controlling factor.

The protein content of seagrasses, marsh plants, and the detritus derived from them has frequently been calculated as 6.25 × nitrogen content (Burkholder et al. 1959; de la Cruz 1965; Odum and de la Cruz 1967; Udell et al. 1969). The data in Table 2 from our method for distinguishing protein nitrogen from nonprotein nitrogen show that the crude method overestimated protein by as much as 180%. In young, green eelgrass leaves the nonprotein nitrogen (in low molecular weight, soluble compounds) accounted for nearly ⅔ of the total, but in senescing leaves the nonprotein fraction was low and the crude protein method overestimated protein by only 10%. Similar analyses of aquatic macrophytes (Boyd 1970) showed that the crude protein method generally overestimated true protein by 10–20%. Our technique differed from that of the earlier studies in that we used a dry combustion method (CHN Analyzer) instead of the traditional Kjeldahl nitrogen determination. The precision of our results (SD of three replicate analyses = ±0.1%) compared favorably with the Kjeldahl method (Morris et al. 1968). Although we did not compare the two methods, another similar dry combustion technique does give data that agree with the Kjeldahl results (Ebling 1968). Total nitrogen content is easily measured, but remains often an inaccurate estimate of the protein content of leaves.

The idea that decomposing leaves contain as much protein as fresh, green leaves (Boysen-Jensen 1914) is not supported by the present study. Total and soluble organic matter, carbon, and nitrogen all decreased as leaves matured, aged, and died (Fig. 4, 5). Some of the data in Fig. 4 and 5 show large fluctuations over short periods. The graphs are accurate as drawn, however, because each point represents the mean of two to four replicate analyses on a sample of leaves pooled from various parts of the eelgrass bed and 95% confidence limits were generally ± 1–5%.

A typical leaf emerged in late winter and had 90% organic matter, over 45% soluble organic

TABLE 2. Leaf protein based on total nitrogen and on TCA-insoluble nitrogen (expressed as percent of dry weight).

Date	Leaf class	Total N × 6.25	TCA-insol. N × 6.25	Nonprotein N as % of total
Mar. 1	Green	28.2	10.6	62.4
	Old	16.5	11.0	33.4
	Dead, in shallow water	10.9	5.1	53.3
Aug. 17	Old	5.3	3.9	26.4
Sept. 20	Old	4.4	4.0	10.0

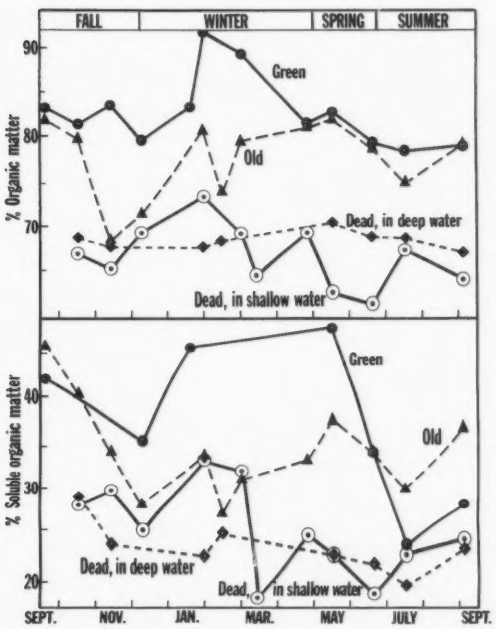

FIG. 4. Total organic matter and soluble organic fraction expressed as a percent of dry weight for each class of leaf, at different times of year.

matter, 42% carbon, and 4.8% nitrogen (all on a dry weight basis). All these values were maxima for the yearly cycle (Fig. 4, 5). As the leaf matured in spring and summer its organic content dropped and remained steady at 80–82%. Part of the decrease may have been due to the observed growth of epiphytic, ash-rich diatoms. At this time the soluble organic fraction extracted reached a minimum of 23%; the mature cell walls reduced the amount of soluble compounds our technique could remove. Carbon decreased somewhat in the mature leaf and fluctuated between 35 and 40% of dry weight. It was nitrogen, however, that changed most rapidly. At the end of summer the mature green leaf had only 1.5% nitrogen, less than ⅓ of the maximum.

An aging leaf differed little, chemically, from a green leaf during summer. The older leaf had slightly less total organic matter, carbon, and nitrogen, but its cell walls were degenerating and hence its extractable (soluble) organic content rose to 45% of the dry weight of the leaf. When leaves became senescent in September they rapidly lost organic matter, the total and soluble organic matter contents dropping to 70 and 28%, respectively. Carbon reached a minimum of 30% at the time of leaf-fall. Nitrogen content, however, remained relatively constant, in contrast to senescing land plants in which nitrogen decreases (Malaviya 1965; Goss 1973). The material lost from the leaf may have leached into the surrounding water, but some of this "food" was probably translocated and stored in the rhizome in preparation for spring leaf growth. Carbohydrate storage was suggested by McRoy (1970b) on the basis of caloric values of rhizomes. We have analyzed the rhizome in winter (using the phenol–sulphuric acid test of Dubois et al. 1954) and found that 33% of the dry weight or 60% of the soluble fraction was carbohydrate.

The dead leaf began, then, with low levels of total and soluble organic matter, carbon, and nitrogen. All four components showed some increase during early winter when old leaves (with higher organic content) were still being recruited into the pool of dead leaves. In late winter the dead leaf lost more of its soluble components, the fraction most easily leached and decomposed, but for the rest of the year the leaf retained about 20% soluble organic matter. Total organic content, carbon, and nitrogen tended to decrease slightly over the year. Contrary to an older report (Boysen-Jensen 1914) nitrogen did not increase even when the dead leaf began to break up,

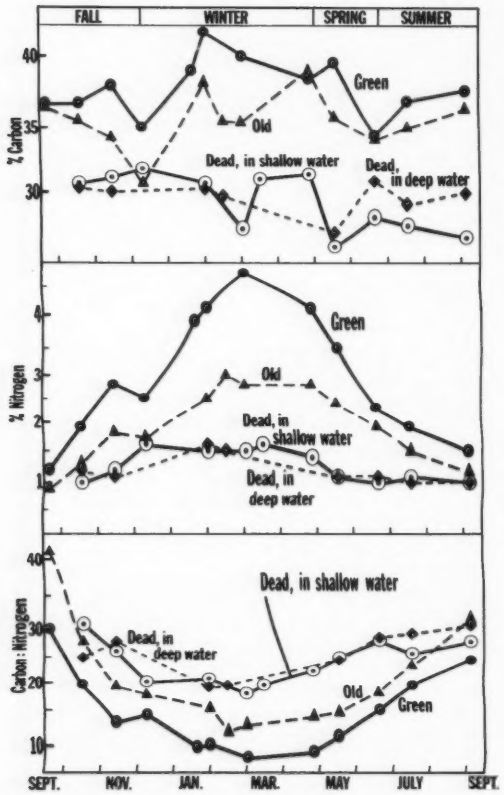

FIG. 5. Carbon and nitrogen expressed as a percent of dry weight for each class of leaf, and the ratio of carbon to nitrogen, at different times of year.

although laboratory decay experiments (Fenchel 1973) indicate that nitrogen may accumulate relative to other components as small leaf fragments decompose. The anomalous slight rise in total organic matter content that occurred when a dead leaf was transported to deeper water could be explained by the observed decrease in abundance of the ash-rich epiphytic bryozoans and calcareous red algae on leaves in deep water.

As an index of seasonal changes in the nutritional value of eelgrass the carbon to nitrogen ratio (C:N) is less informative than either the carbon or nitrogen data alone. Because of the large variations in nitrogen and the relative stability of carbon, the C:N curves simply reflect changes in nitrogen. If 17:1 is the maximum C:N for good animal nutrition (Russell-Hunter 1970, p. 174–182), then green and old leaves were potential food for grazers during winter and spring (Fig. 5). Interestingly, only during summer, when even green leaves had a C:N above 17:1 (up to 30:1), did any animal eat the leaves. The snail *Lacuna vincta* (Littorinacea) was common in summer on green leaves (1–3 snails/plant) and it did consume a small amount of leaf (confirmed in laboratory experiments) but its main food was the epiphytic diatoms. The C:N ratio is not the only factor influencing the nutritional value of eelgrass.

Acknowledgments

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System Simulation of the Predatory Activities of Sea Lampreys (*Petromyzon marinus*) on Lake Trout (*Salvelinus namaycush*)

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A stochastic dynamic model was developed to evaluate the simulated and empirical interactions between sea lampreys (*Petromyzon marinus*) and two lake trout (*Salvelinus namaycush*) population structures, one without and the other with previous lamprey predation.

An arithmetic increase in lampreys induced a geometric decline in trout, which was dependent on the number of age-classes and the mean weight of trout. Large trout, when present, were subject to most of the lamprey predation allowing survival of smaller trout. Lethality increased exponentially with lamprey size resulting in peak lake trout mortality during fall near the end of the lamprey's feeding period. Lake trout scarring data could be used only as an index of lamprey abundance when trout:lamprey ratios and trout population structures were known. The simulation suggested that sea lampreys and lake trout could coexist if large trout are not removed by commercial fishing and if some exogenous factor controlled lamprey abundance.

LETT, P. F., F. W. H. BEAMISH, AND G. J. FARMER. 1975. System simulation of the predatory activities of sea lampreys (*Petromyzon marinus*) on lake trout (*Salvelinus namaycush*). J. Fish. Res. Board Can. 32: 623–631.

Les auteurs élaborent un modèle dynamique stochastique permettant d'évaluer les interactions simulées ou empiriques entre la lamproie marine (*Petromyzon marinus*) et deux structures de population de touladis (*Salvelinus namaycush*), l'une ayant échappée à une prédation antérieure par la lamproie marine et l'autre l'ayant subie.

Une augmentation arithmétique du nombre de lamproies provoque une diminution géométrique du nombre de touladis, qui dépend du nombre de classes d'âge et du poids moyen du touladi. Lorsque présents, les grands touladis sont sujets à la plus grande partie de la prédation par la lamproie marine, ce qui permet aux petits touladis de survivre. La mortalité augmente en fonction exponentielle de la taille de la lamproie, ce qui résulte en un pic de mortalité des touladis en automne, alors que la période d'alimentation de la lamproie tire à sa fin. Il a été possible d'utiliser les cicatrices des touladis comme indice d'abondance des lamproies seulement quand les rapports touladis: lamproies et la structure des populations de touladis étaient connus. La simulation suggère que lamproies marines et touladis peuvent coexister si les grands touladis ne sont pas enlevés par une pêche commerciale et si un facteur exogène quelconque contrôle l'abondance des lamproies.

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COINCIDENT with the movement of sea lampreys (*Petromyzon marinus*) into the upper Great Lakes of North America was a dramatic decline in the abundance of some teleosts (Creaser 1932; Applegate 1947; Shetter 1949). The species whose numbers were most severely reduced were lake trout (*Salvelinus namaycush*), lake whitefish (*Coregonus clupeaformis*), burbot (*Lota lota*), and several of the smaller species of whitefish (Smith 1971). This was followed by a reduction in the numbers of medium-sized, deepwater species and larger species from shallower depths. There is evidence indicating that the decline of lake trout stocks began when the abundance of sea lampreys was low (Smith 1968), suggesting

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that some factor in addition to lamprey predation was responsible. In Lake Michigan, Smith (1968) suggests that the abrupt decline of the lake trout population reflected the combined effects of increased lamprey predation and greater exploitation by the commercial fishery. The question of whether the fish stocks would have declined to the same extent in the absence of a commercial fishery remains unanswered. However, it is known that sea lampreys and lake trout cohabited Lake Ontario, Seneca Lake, and Cayuga Lake for centuries in the absence of fishing pressure.

Budd et al. (1969) describe the effects of sea lamprey predation on the lake trout population of South Bay, Lake Huron. Because a quantitative estimate was available for lamprey inflicted mortality, a unique opportunity was provided to test a system simulation describing the predatory interactions between the two species. The aim of the simulation was to estimate the ratios of lampreys and lake trout in the Great Lakes that would allow cohabitation and the type of trout population structure best facilitating cohabitation.

The Program

The program, written in General Purpose System Simulation (G.P.S.S. 111) was run on an I.B.M. 370 computer. This language is particularly adept for simulating predator–prey interactions due to the presence of an internal clock which serves as the independent variable for time dependent processes. As individual fish are simulated, the random or stochastic nature of a biological system may also be expressed as both discrete and continuous events. The program requires approximately 140 K bytes and 15 min of C.P.U. time. The systems flow chart is available from P.F.L.

Structure of the Model

The model was based on a 210 daytime interval extending from May to December because most lamprey growth occurs during this period (Applegate 1950). The unit of time was hours allowing time dependent relationships such as growth and mortality to be described by iterative addition of hours and days. Lake trout was the only host species used in the simulation because data permitting incorporation of other teleosts were not available. Ideally the system should include those species found in association with trout.

The lake trout population structure used in the simulation was that observed during 1954 in South Bay, Lake Huron (Budd et al. 1969) and was comprised of immature individuals of up to 6 yr (Fig. 1). These data were used to describe the structure of the hypothetical unexploited population (Fig. 1). Furthermore, it presumes that the distribution of lake

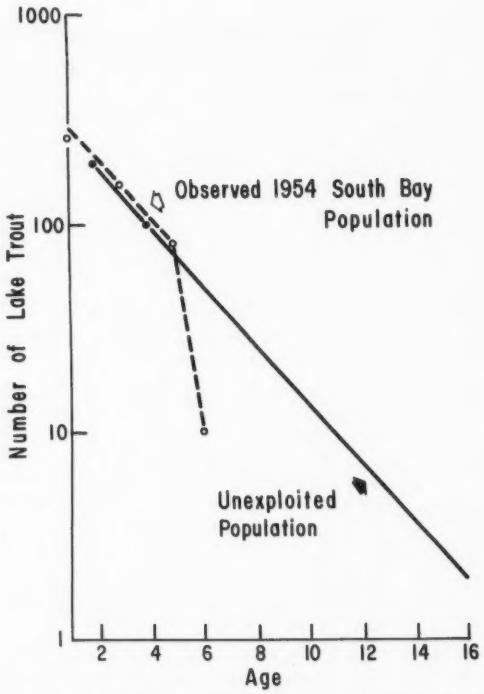

FIG. 1. Age-class structure of the two lake trout (*Salvelinus namaycush*) populations used in the simulation. The structure of the unexploited population was derived by extrapolation from the structure observed for the lake trout in South Bay during 1954 (Budd et al. 1969).

trout throughout the test period is such that the smallest lamprey can locate at least one potential host within a span of 2 days. In the model, as the trout population is reduced by mortality, the mean distance between fish was assumed to remain constant as compensations are made by reductions in the outer periphery of the concentration. Presumably the time needed to make these compensations will increase as the number of trout is reduced. This increment may partially be balanced by an increase in swimming performance of sea lampreys as they grow larger (Beamish 1974). Although the model allows for seasonal variations in lake trout density, a precise description of these movements is not available for application at this time.

Age was assigned stochastically to each trout in the simulation and was assumed to be normally distributed with variability increasing with age (Fry 1949), (Fig. 2A).

Sea lampreys given a choice of teleosts of different weights but of the same species select the largest individuals most frequently (Farmer and Beamish 1973). Although Farmer and Beamish (1973) deter-

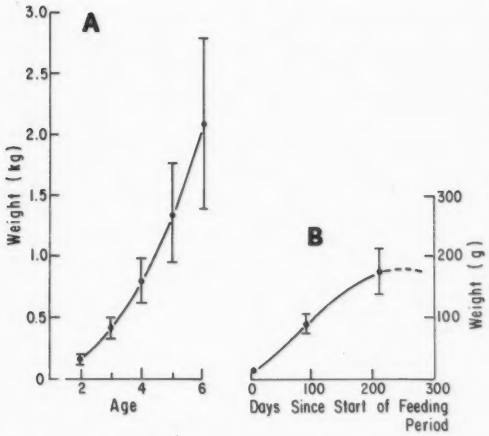

FIG. 2A. Relation between age and weight for sea lampreys (*Petromyzon marinus*) from South Bay, Lake Huron (Budd and Fry 1957; Carlander 1969). Bars denote 1 SD. B, Weight gain of sea lampreys during the simulated feeding period of 210 days (from Applegate 1950). Bars denote 1 SD.

mined that the relationship between priority of attack and teleost weight was positive and linear, little is known about the accuracy of the mechanism by which lamprey differentiate among fish weights. Thus, the priority of attack under ecological conditions is a probability but, until more information becomes available, a deterministic priority of attack was assigned each fish based on their weight. In this context, B. G. H. Johnson (personal communication) observed that, when large trout were available in the Great Lakes, lampreys generally associate with them rather than with smaller individuals. The host weight priority was in effect only after all lampreys had attacked for the first time, all subsequent attacks after the first being on the largest trout. This inadequacy of the model proved of little consequence because lamprey initially weigh 10 g and are not able to kill even the smallest trout at this time. The number of lamprey attacks a trout received at a time was limited to four in accord with laboratory studies which demonstrated that the pattern of multiple lamprey attacks followed a binomial distribution truncated below and above four (Farmer and Beamish 1973).

Trout can be preyed upon in the model only when lampreys are available. However, the availability of one or more sea lampreys does not imply a trout will enter the feeding section of the model unless its number of predicted attacks is equal to or less than the number of free potential predators. On entry into the feeding section, a trout moves into a storage of simulated lampreys of various weights feeding simultaneously on hosts of different age and size. However, lamprey feeding together on the same lake trout are of equal size, and attack and detach at the same time. Attachments are not concurrent under field conditions, but detachments from dead trout would occur nearly simultaneously. Presumably, on average, simultaneous attachment has little effect on the resultant loss to the trout population.

Lampreys not attached in this area are then able to search for newly arrived trout. As teleosts apparently make no effort to avoid lampreys (Farmer and Beamish 1973), the search is facilitated by freezing the new trout's movement for a stochastic time period with a mean of 2 days and a normally distributed standard deviation (SD) of 9 h. The selection of the time interval between successive feedings was based on the concept that rate of food passage through the gut determines appetite (Brett 1971). In this context, Farmer (unpublished data) found that the guts of satiated lampreys held at 10 C were empty within 4 days. As lampreys spend little time between periods of feeding, the effects of systemic need would be minimal as a motivation for searching.

After association with a particular trout, a lamprey is assigned a weight following a growth pattern calculated for each lamprey from the data collected for lampreys in Lake Huron (Applegate 1950) (Fig. 2B). A new weight was assigned to lampreys when they attacked new hosts, the increasing variance in lamprey weight being accounted for by multiplying the SD of weight by a value randomly determined from a standard normal distribution. The value was then added to the mean weight also calculated as an increasing function of age.

Lampreys do not always remain attached to a fish until mortality occurs. Laboratory observations (Farmer unpublished data) have shown that mean attachment time, not resulting in death, is about 10 days, although the exact nature of the distribution is not well understood. Until further information becomes available, we have assumed a normal distribution with five standard deviations to represent a lamprey that attacks and shortly thereafter releases without feeding. The termination of the right-hand limb of the distribution represents lamprey that are attached up to 20 days.

Time to death of trout related to the size of the lampreys feeding on them and the amount of their daily consumption of blood. This relationship was described by Farmer et al. (1974) as: $\log y = 3.311 - 1.533 \log x$ where: x is the amount of blood lost daily by trout expressed as a percentage of their blood volume and y is the time to their death in days. Blood consumption by lampreys at 10 ± 1 C was quite variable, ranging from 2.9 to 29.8% wet body weight/ day with a geometric mean of 11.6 ± 7.5%/ day (Farmer et al. 1974). The blood volume of lake trout was assumed to be 3.125% wet weight as found by Conte et al. (1963) for rainbow trout, *Salmo gairdneri*.

Mortality of the simulated lamprey population was similar to that found for laboratory-held animals (Parker and Lennon 1956), the numbers decreasing from 0.90 of the total during May to 0.38 in December.

It was presumed that both lake trout and sea lamprey remained at 10 C throughout the simulated period of 210 days. McCauley and Tait (1970) found the final preferendum temperature was 11.7 C for lake trout which was 2 C warmer than the temperature at which lake trout are most commonly caught in thermally stratified lakes. McCombie (1967) investigated the seasonal thermal regime of South Bay, Lake Huron, and showed that 10 C water was available to lake trout throughout the feeding period.

While a lamprey is attached to a trout, the host's progress through the model is delayed for the assigned amount of attachment time. If, at the time of release, the trout population has been reduced to a level that would substantially affect the period needed by lampreys to locate a potential host, the trout is required to wait before being able to rejoin the remaining trout. When attachment time is less than the interval necessary to kill the host, the fish leaves the model simulating its death. The survival time, age, and weight of the trout are tabulated before it is removed from the model. Trout that survive are recorded as scarred if the lampreys that fed on them weigh more than 30 g. It was presumed that scars resulting from attachments by lamprey below 30 g would not be detectable by the end of the feeding period. These fish now rejoin the remaining trout waiting entry into the feeding section. If sufficient time remains in the feeding period, many of these marked fish will be attacked a second time and perhaps killed. As the larger trout are frequently attacked (Farmer and Beamish 1973), smaller trout may not be preyed upon. The mean size of fish killed is, therefore, dependent on the abundance and growth rate of sea lampreys. When the feeding period terminated, these remaining fish were transferred to a section of the model where their age, weight, and numbers are tabulated.

The Planted Lake Trout Population of South Bay, Lake Huron

Hatchery lake trout were planted in South Bay of Lake Huron between 1949 and 1953 because sea lampreys had virtually eliminated the native trout population. Experimental sampling during 1954 indicated that lamprey scars were not present on recaptured trout of less than 3 yr, but for those of 3–5 yr scarring increased from 5.2 to 38.8%. In addition, annual mortality of trout less than 4 yr of age was found to be similar to that observed for lake trout populations receiving no lamprey predation (Budd et al. 1968; Paloheimo 1958). The data collected for this population (Budd et al. 1969) allow comparisons with a simulation of the interaction between sea lamprey and lake trout.

Results and Discussion

The simulated lake trout population consisted of 500 individuals (Fig. 1), which provided sufficient fish in the older less abundant year-classes to reduce the chances of aberrant results. The implemented number of lampreys, which was varied between 1–250, represented their preponderance in May, the period when active feeding becomes most pronounced. Trout mortality was 1.00 until the number of lampreys was reduced to 50. With only 17 lampreys mortality of trout approached zero.

The reduction of lake trout numbers in the simulated 1954 stock previously planted in South Bay (Budd et al. 1969) attributable to varying abundance of lamprey followed a sigmoid distribution given by

$$(1)\quad \log_e \frac{(K-N_t)}{N_t} = 2.683-0.106N_L \pm 0.018 \text{ (2 SE)}$$

where N_t is the number of dead trout for each level of N_L
N_L is the number of lamprey at the beginning of the feeding period
K is the upper asymptote of lake trout mortality
the coefficient of determination (r^2) was 0.896.

The instantaneous decrease in trout due to lamprey predation is 0.106 for the 1954 South Bay lake trout population. Mortality, i.e. the total loss of trout due to lamprey predation, remained below 0.20 when *P. marinus* numbered between 0 and 20. A 50% increase in the number of lampreys to 30, a prey:predator ratio of approximately 15:1, simulated a 300% decrease in the number of trout. After this rapid increase, trout mortality began to level off as mortality neared 1.00.

Mortality of trout by age-class also increased with varying numbers of sea lamprey (Fig. 3). Only fish to 5+ are included because not enough age 6+ fish died in the simulated population of 500 fish to give reliable estimates of mortality. Each age-class experiences double exponential increase in mortality with increasing lamprey abundance, but the rate of increase of the relationship varies with age. This relationship is double exponential due to the additive exponential relationships of blood loss and multiple attack. Christie (1974) presents field data showing an exponential decline for 3+ lake trout. Therefore, a dramatic decline in lake trout with small increase in lamprey numbers is dependent on the age or size structure of the fish stock. The rate of decline of younger fish was of course greater than that for older fish. When 17 (ratio = 30:1) lamprey are present initially, mortality of 5+ lake trout is 0.40 but for 2-yr-olds mortality is only 0.02. When lamprey abundance is 30, lake trout mortality for both 2+ and 5+ trout is approaching 1.00.

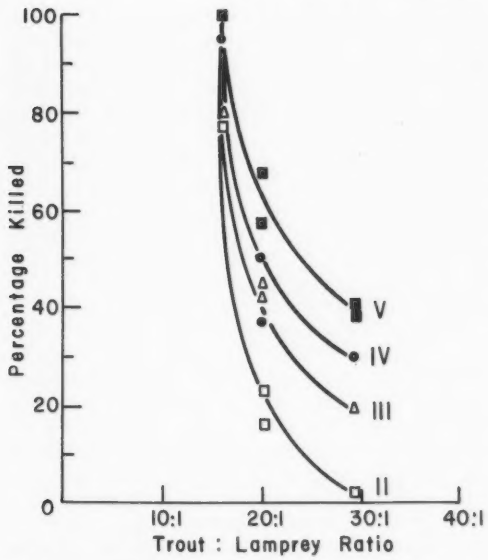

FIG. 3. Age specific mortality of lake trout induced by varying lamprey abundance.

Coincident with shifts in age specific mortality due to changing lamprey abundance were fluctuations in trout survival. The relationship between survival, lamprey abundance, and trout age is illustrated in Fig. 4 and equation 2 for the planted lake trout population of South Bay in 1954.

(2) $N_s = 20.77X_1 + 16.97X_2 - 4.00X_1X_2 + 0.209X_1^2X_2 - 130.01$

N_s is the number of survivors at the end of the feeding period
X_1 is the year-class of lake trout
X_2 is the ratio of lake trout to lamprey

the coefficient of multiple determination (r^2) = 0.856.

When lampreys number 17 (ratio = 30:1), the greatest impact of predation was taken by 4+ and older trout; however, scarring was replaced by a mortality for 3-yr-olds. Consequently, although lamprey fed more heavily on the older fish, the younger fish suffered a higher mortality, because they can be killed more easily. As the abundance of lamprey increase there is a tendency toward fewer younger survivors. Eventually the highest mortality shifts to the age 2+. Trends in age specific mortality with varying lamprey abundance are also reflected by the seasonal onset of noticeable predation.

The total number of fish killed each month was

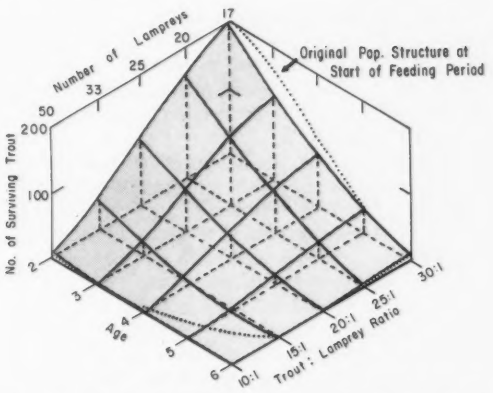

FIG. 4. Relation between trout:lamprey ratio and survival among the various trout year-classes.

determined by varying the cumulative number of days that lamprey were feeding and the abundance of lamprey. The results of this manipulation appear in equation 3 and Fig. 5.

(3) $N_m = 1.87X_2 + 3.28X_1^2 - 2.2 \times 10^{-4}X_1^3 - 3.39 \times 10^{-1}X_1X_2 + 9.35 \times 10^{-3}X_1X_2^2 - 4.0 \times 10^{-5}X_2^2 + 1.08 \times 10^{-5}X_1^3X_2 - 1.08 \times 10^{-7}X_1^3X_2^2 + 4.89$

where N_m is the monthly total of dead lake trout
X_1 is the cumulative number of days lamprey have been feeding
X_2 is the ratio of lake trout to lamprey existing at the beginning of the feeding period

the coefficient of multiple determination (r^2) is 0.843.

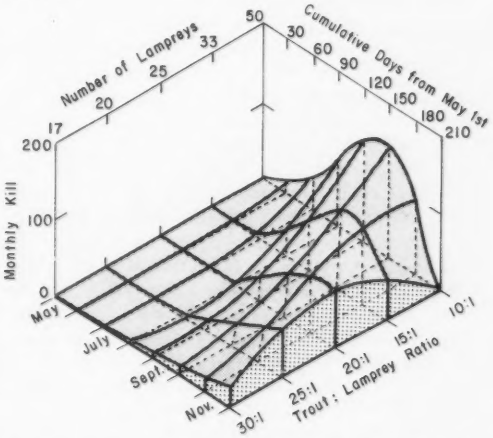

FIG. 5. Influence of lamprey abundance on number of trout killed monthly from May to December. Initial trout population was 500.

With an initial abundance of 50 lamprey, mortality of trout was highest between September and October and declined to zero by December, the latter resulting from a decrease in trout numbers. More trout could have been killed had they been available.

As the number of lampreys decreased, the highest monthly mortality of trout moved from September to late November (Fig. 5). Coincident with this shift, the first indications of mortality transfer from early spring to late summer.

When lampreys were abundant, the death of trout was first noticed in the early summer, a result of the increase of multiple attacks by lampreys despite their small size, 50 ± 20 g, at this time. As the initial number of lampreys in a population declines to less than 50, the incidence of multiple attacks and mortality of trout decreased, the latter exponentially. Single attacks by small *P. marinus* seldom killed the host, particularly those of the larger size preferred by sea lampreys (Farmer and Beamish 1973). Calculated time to death from a single attack by a lamprey initially of 30 g on a trout 5–10 kg was longer than the period of active feeding, whereas 30–100 days were required by a 150-g *P. marinus* to kill the same trout. In contrast, lampreys of 30 g can kill trout of 0.5–1.0 kg in only 5–15 days. Resistance by preferred large trout, therefore, increased survival of the smaller individuals.

The buffering effect by large trout was investigated by placing into the model a stock of 500 unexploited fish, age structured by extrapolation of the semilogarithmic regression of lake trout frequency and age from 6+ to 13+ (Fig. 1). This of course assumed identical instantaneous mortality between 2+ and 5+ in the two stocks. With a stock of 500 trout the probability of individuals over 13+ was sufficiently low to exclude them from the model. Trout between age 5+ and 13+ represented 10.8% of the fish in the population. Mean weight of each age-class above 6 was estimated by extrapolation of the regression line described by Budd and Fry (1957) for the South Bay trout and remained normally distributed (Fry 1949) as selective exploitation did not occur.

The mortality and scarring frequency observed for trout in South Bay during 1954–56 (Budd et al. 1969) was best approximated in the simulated exploitated stock exposed to 25 lampreys, a prey:predator ratio of 20:1. For comparison, lampreys were introduced into the unexploited stock of 50 lake trout. Mortality of trout on addition of sea lampreys to the previously unexploited population was 0.22 ± 0.08 (SD), appreciably less than the 0.35 ± 0.04 for the simulated population having the South Bay age-structure. This reduced level of mortality approximates that found in stocks of lake trout that had suffered minimal exploitation (Budd et al. 1968; Paloheimo 1958). The average weight of fish killed was 0.98 kg when the population included trout up to age 13 whose mean weight was 12 kg. In sharp contrast, the average weight of trout killed by lampreys in the simulated South Bay population was 0.59 kg.

Indices of sea lamprey abundance in 1954–55 were about half those observed for peak numbers in Lake Huron for 1949 (Smith 1968; 1972). The resistance of the unexploited trout population to predation at this peak abundance was determined by doubling the number of simulated lamprey, thereby producing a prey:predator ratio of 10:1. This resulted in a total mortality of 0.41 ± 0.08. When the ratio of trout to lamprey was 20:1, the brunt of predation was taken by individuals over the age of 7+, but the majority of these fish survived with scars (Fig. 6). At a lamprey abundance of 50, a prey:predator ratio of 10:1, these older fish would be killed and few would survive beyond the age of 6. This ratio had previously led to almost total extinction of the South Bay planted population (Fig. 4) with no trout surviving past age 3+.

The simulated results make no distinction between a wound and a scar. The observed number of scars by age in 1955–56 for the 1954 South Bay population was compared with simulated scarring rates when the ratio of lake trout to lamprey was 20:1. This ratio produced mortality similar to that observed by Budd et al. (1969), a χ^2 test for goodness of fit (Steel and Torrie 1960) showing nonsignificant difference at $P \leqslant 0.05$.

The simulated incidence of scarring on older fish tended to increase as lamprey abundance decreased, but this trend was not statistically significant. When 50 lamprey were present, the total percentage of marked fish was approaching zero, because at this ratio (10:1) almost the entire population of trout was killed. As the number of lamprey decreased to 25, the scarring rate increased to a level of 11.3% as more older fish survived. At a still lower level of lamprey abundance there was a marked decline in simulated scarring to 8.6% for a lake trout to lamprey ratio of 30:1.

The more resistant the population was to lamprey predation due to older and larger fish, the higher the scarring rate. Percentage scarred of all trout at a ratio of 20:1 for the 1954 South Bay

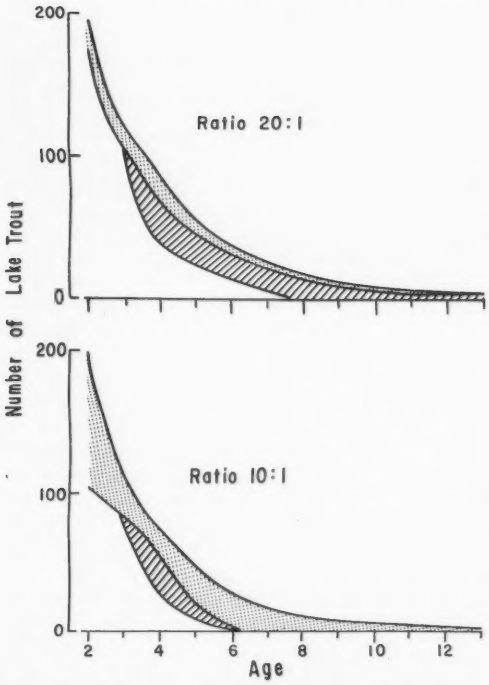

FIG. 6. Influence of trout:lamprey ratio on mortality in the unexploited trout population. Stippled area represents numbers of trout killed and striped area the number of fish that survived but bore scars. Upper line indicates the numbers of trout in the various age-classes at the beginning of the 210 day feeding period.

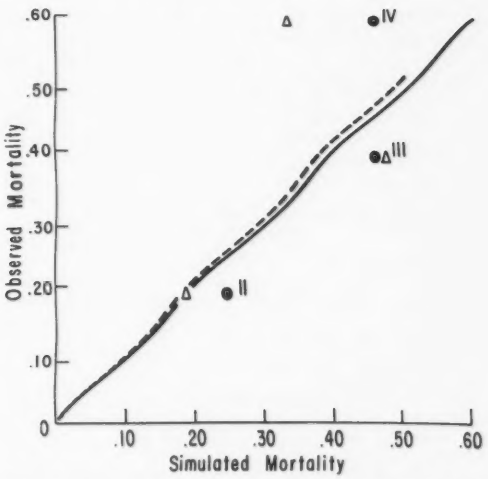

FIG. 7. Relation between natural mortality of 2-, 3-, and 4-yr-old lake trout in South Bay (Budd et al. 1969) and mortality values produced by the simulation for these year-classes. The 45° angle represents parity between observed and simulated mortalities. Triangle and circle represent two runs of the simulation using different seeds in the random number generator.

population was 10.3%, whereas for the population structure including older individuals the scarring rate increased to 17.6%.

Natural mortality (mortality from all causes with the exception of fishing) was estimated by Budd et al. (1969) for 2+, 3+ and 4+ lake trout. These values obtained from field studies best fitted simulated mortalities caused by 25 lamprey or a prey:predator ratio of 20:1 (Fig. 7). A line at a 45° angle from the origin was drawn between the axis of natural mortality and simulated lamprey mortality to illustrate parity between the two estimated factors. A regression line was fitted to the data to determine the trend between observed and predicted values of mortality.

(4) $\log_{10}M_s = -0.011 + 1.017 \log_{10}M_p \pm 0.418$ (2 SE)
where M_s is the simulated mortality
M_p is the observed mortality

The regression through these points is nonlinear and indicates that older fish are beginning to survive sea lamprey predation slightly beyond the calculated values of natural mortality, this being accompanied by an increase in scarring. It would thus seem that the reduction in stock size accompanying lamprey predation decreases mortality caused by factors other than *P. marinus* to zero — a compensatory trend observed in most commercial fisheries which serves to reduce the environmental pressure on survivors (Royce 1972). This compensation was especially true for the lake trout of year-classes 2+ and 3+ that could not resist at least one lamprey attack (Farmer 1974). Budd et al. (1969) provided examples ilustrating that this mortality did not differ from that of other lake trout stocks. Both the simulated results and field observation suggested that no scarred younger trout were recovered. Lack of scarring was an indication that these year-classes are heavily preyed upon and that all attacked fish died. This level of predation completely reduced mortality from other causes.

Scarring frequency was useful as an index of lamprey abundance only when substantial numbers of trout within a population were large enough to survive predation and when mortality

increased throughout the lamprey feeding period. Interpretation of scarring data has, in the past, been much of an enigma. For example, Christie (1972) found a negative correlation between wounding and size of lake whitefish, *C. clupeaformis*, whereas Budd et al. (1969) observed a positive correlation between scarring rate and size of lake trout.

The reduction in mortality from the addition of older lake trout tends to indicate that these fish could coexist with sea lampreys. Although mortality was relatively high (0.41 ± 0.08) when the 50 lamprey were added to the unexploited population, this population structure included fish only to the age of 13^+. It is not uncommon to find trout in truly virgin populations over the age of 20^+. Also, the unexploited population structure followed a type II pattern (Krebs 1972). In contrast, Kennedy (1954) demonstrated a type I curve is more likely for unexploited populations of *Salvelinus namaycush*. In any case, coexistence with lake trout is enhanced by any factor that can limit lamprey abundance. There is some evidence that *P. marinus* and lake trout indeed were concomitant in Lake Ontario from at least as early as 1835 (Lark 1972). Christie (1972) suggests lamprey abundance increased in this region during the last century in association with the deterioration of impoundments on tributaries. During the period 1915–45 commercial exploitation undoubtedly removed substantial numbers of the larger lake trout from the population, especially as fishermen were allowed to set nets on spawning beds (S. Rankin personal communication). Thus, the increased abundance of sea lampreys together with the smaller number of large trout presumably contributed largely to the well-documented collapse of *S. namaycush* in Lake Ontario (Christie 1972).

Commercial catches of lake trout in Lake Huron were declining despite increases in fishing effort between 1890 and 1937 (Berst and Spangler 1972). In the early 1940s sea lampreys had only recently invaded the upper Great Lakes. The rapid decline of lake trout stocks at this time occurred at abundance levels of lamprey far less than that at their peak (Smith 1972). Assuming that the numbers of lampreys in South Bay reflected changes of abundance in the rest of Lake Huron, it is wrong to suppose that the collapse of stocks of lake trout was entirely due to sea lampreys. The simulated results for the unexploited population indicate lamprey numbers analagous to the 1954–55 abundance levels could not cause mortality serious enough that small trout would be killed before maturing, thereby causing a significant drop in recruitment.

That the model demonstrates a geometric decline in the number of trout with arithmetic increments in lamprey abundance (equation 1) is an important outcome, because it suggests that a high variance in lamprey numbers, produced by strong year-classes followed by a number of years of lower abundance, would increase the probability of survival of a lake trout population. However, a stable, continual increase in lampreys eventually must result in the collapse of a lake trout stock. Support for this hypothesis is found in Cayuga Lake which contains a lake trout population that coexists with a fluctuating lamprey population (Wigley 1959). It is also possible that a reduction in lake trout numbers would retard the reproductive potential of sea lamprey in Cayuga Lake but, as other species serve as alternate prey (Youngs and Oglesby 1972), lampreys would be slow to respond to reductions in stocks of favored species. Possibly some exogenous factor, such as low water temperature at the time of spawning, may be affecting subsequent lamprey abundance. Low water temperature has been observed to severely reduce spawn activity (Wigley 1959).

Total mortality of lake trout in Cayuga Lake annually varies between 0.25 and 0.60, whereas minimum values estimated for fishing mortality are 0.04 and 0.10 (Youngs and Oglesby 1972). Although it is suggested fishing mortality could be higher, the exogenous control of temperature over sea lamprey abundance is a possible key factor determining total mortality.

In Cayuga Lake lampreys spawn principally in one tributary. Hence unfavorable water temperatures, which affect spawning success, would presumably reduce recruitment of juvenile lampreys although transformation of larval stages of the same age over several years would tend to dampen this effect. In contrast, at least some of the large numbers of tributaries of the Great Lakes would undoubtedly provide optimum temperatures for reproductive success in any given year. In this way recruitment of juvenile lamprey is perhaps more variable than in the upper Great Lakes.

Because fishing mortality in Cayuga Lake results only from a sports fishery operating at a low level (Youngs and Oglesby 1972), the probability of the larger trout surviving is greater. The effect of these older, larger fish acting as a buffer, coupled with varying lamprey recruitment, may be the best explanation of the coexistence of sea lamprey and lake trout in this area.

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Spawning Behavior of Chum Salmon (*Oncorhynchus keta*) and Rainbow Trout (*Salmo gairdneri*)

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TAUTZ, A. F., AND C. GROOT. 1975. Spawning behavior of chum salmon (*Oncorhynchus keta*) and rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 633–642.

Detailed accounts of the spawning behavior of chum salmon (*Oncorhynchus keta*) and rainbow trout (*Salmo gairdneri*) in laboratory flumes are provided. The behavioral activities, quivering, probing, and crossing over increase in frequency as a function of time prior to spawning whereas digging remains constant or decreases slightly. Maps of digging locations and movie films suggest nest shape and current pattern are monitored by the female, allowing her to intensify her digging activity near the center of the nest. Probing appears to be a signal to the male indicating approach of oviposition and also provides information to the female regarding the shape and suitability of the nest site. The probing act is also used in the synchronization of the male and female spawning acts. Changes in velocity do not appear to markedly affect nest construction either in terms of number of digs to oviposition or in size of nest constructed. It is inferred that velocity and gravel size are important insofar as they influence the construction of a suitable nest depression. Nest sites would appear to be selected on the basis of acceleration of flow rather than velocity per se though high limits must obviously exist.

TAUTZ, A. F., AND C. GROOT. 1975. Spawning behavior of chum salmon (*Oncorhynchus keta*) and rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 633–642.

Nous donnons un compte rendu détaillé du comportement reproducteur du saumon keta (*Oncorhynchus keta*) et de la truite arc-en-ciel (*Salmo gairdneri*) dans des canaux en laboratoire. Les activités du comportement, frémissements, sondages et traversées augmentent de fréquence en fonction du temps qui sépare les poissons de la fraie, alors que le creusage demeure constant ou diminue légèrement. Des cartes indiquant les endroits de creusage et des pellicules cinématographiques suggèrent que la forme du nid et le courant sont surveillés par la femelle, ce qui lui permet d'intensifier son creusage près du centre du nid. Le sondage semble être un signal adressé au mâle pour indiquer l'approche de l'oviposition; il renseigne également la femelle sur la forme et la convenance du site du nid. Le sondage est aussi un acte utilisé pour synchroniser la fraie du mâle et de la femelle. Les changements de vitesse du courant ne semblent pas affecter de façon appréciable la nidification, en termes ni du nombre de creusages précédant l'oviposition ni de la grandeur du nid. On en déduit que la vitesse du courant et la grosseur du gravier sont importants en autant qu'ils affectent la construction d'une dépression convenable pour le nid. Les sites de nidification seraient apparamment choisis sur la base de l'accélération du débit plutôt que de la vitesse per se, bien qu'il doive évidemment exister des limites supérieures.

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As fisheries biology moves towards the implementation of intensive fisheries management programs, the development of criteria used in the definition and estimation of relevant population dynamics parameters becomes progressively more important. In the case of estimates of spawning area for salmonid fishes, velocity and gravel composition would appear to be of some consequence in the definition of such criteria, but the exact nature of the interaction between the spawner and its environment is not well understood. This paper provides some preliminary ethological observations useful for the development of a classification of spawning environments for salmonid fishes. Included are the identification, quantification, and comparison of behavior sequences prerequisite to spawning for chum salmon (*Oncorhynchus keta*) and rainbow trout (*Salmo gairdneri*), as well as observations on the effects of altered velocity on spawning parameters such as nest shape, size, and ease of nest construction.

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Materials and Methods

Chum Salmon

Pairs (65–75 cm FL) were isolated in a stream flume during October 15, 1970 to January 1, 1971, at Big Qualicum River on the east coast of Vancouver Island, B.C.

The apparatus (Fig. 1A) consisted of a 4.9 × 1.2 m plywood flume with a 1.8-m periscope built into one side. The front of the periscope box consisted of a 1.8 × 0.6 m piece of 1.3-cm (thick) plate glass and inside were placed two 1.8 × 0.3 m mirrors. Neutral buoyancy was obtained by partially filling the periscope with water. An observer was thus able to view spawning activity at an apparent underwater distance of 30–60 cm from the spawning pair, and so long as the viewing area was darkened, was able to move about without disturbing the fish. The apparatus was placed in an unused rearing channel supplied with water from the main river system. A small dam across the width of the channel created a head sufficient to produce desired velocities.

For each experiment a mature male and female were captured during upstream migration in a large trap and counting fence facility. The pair was transported to the flume in a tank on the back of a pickup truck within 10–15 min.

Records of digging activity were obtained by combining an LC-10 transducer (Atlantic Research Corp. Ser–1286), a Uher tape recorder, an Acoustomat trigger, and a Rustak 4 channel event recorder. The equipment could function unattended in excess of 24 h and would accurately record digging activity under almost all circumstances. Velocities during spawning fluctuated between 25 and 60 cm/s with changes in river level, whereas temperature (8 C) and photoperiod (9 light – 15 dark) were those experienced by the fish under natural conditions.

Rainbow Trout

Experiments were conducted from April 5 to June 5, 1971, at the Fisheries and Marine Service Laboratory, Nanaimo, B.C. Twelve pairs of trout (23.5–32.5 cm FL) were obtained in late April from Loon Lake Outlet (described in Hartman et al. 1962), and transported to Nanaimo in an aluminum holding tank. Water was periodically reoxygenated during transport with airstones connected to an oxygen cylinder, and temperature was maintained between 6 and 10 C by addition of crushed ice.

Trout were kept outdoors in a circular fiberglass tank for up to 30 days until mature. Hatchery food was added, but no feeding was observed. No attempt was made to duplicate water temperatures at Loon Lake Outlet, which vary dramatically during the course of the spawning season (Hartman et al. 1962). The fish were exposed to normal Nanaimo (50 C) photoperiods for the season, which do not differ much from those at Loon Lake (51°lat.).

The experimental apparatus consisted of a 7.0 × 2.4 × 1.2 m tank with plate glass windows along one side, divided lengthways into two channels to provide a continuous flow of water. Fish were restricted by net partitions to the area near the glass window, which contained a gravel box for spawning, a ramp, and pool area (Fig. 1B). Water depth was maintained at 20 cm over the gravel and 35 cm in the rest of the tank. A continuous supply of fresh water maintained temperature at 14 C, well within the range of normal spawning conditions.

Velocities were more controlled than in the chum experiments. Two 2-hp enclosed impeller pumps were used singly or together to produce two flow regimes. Water from each pump was directed to a manifold containing a series of 1.4-cm diameter nozzles. With two pumps, turbulent velocities of 60 cm/s and laminar velocities of 37 cm/s were recorded. With one pump, laminar flows of 24 cm/s were obtained. Flows experienced by the fish during selection and early construction of the nest were measured 5 cm from the bottom with an Ott current meter.

Gravel consisted of commercially available "torpedo" gravel (9 mm diam) over which 40 larger rocks of different sizes (12–50 mm diam) were arranged in a grid pattern. These rocks were painted various colors to aid in observing how gravel particles moved during the construction of the nest.

Data on the particle movements and dig locations were recorded through use of a mirror suspended at 45° over the gravel area, and a Bolex 16-mm film camera with single frame exposure capability. Other behavioral activities were recorded on an Estraline–Angus 20 channel event recorder. Films were analyzed with the aid of a Vanguard Motion Analyzer (Model m-16 CW).

To initiate an experiment, a pair of mature fish was placed in the flume. If no spawning activity was observed within 3–4 h, the fish were returned to the holding facility and the procedure repeated, usually the next day. Because variation in behavior from female to female was potentially large, each female was allowed to construct two nests, one under high and the other under a low velocity regime. Assignment of a high or low velocity for the first nest was alternated for each successive pair, and for each pair the switch from high to low or low to high occurred 50 digs after the first oviposition.

After two spawnings, the pair was removed. Eggs deposited in the gravel were excavated the next morning and after levelling the gravel area and filling the tank with fresh water, a new pair was introduced.

Results

Basic Behavior

Spawning behavior in both chum salmon and rainbow trout consists of a combination of nest building by the female and courtship display by the male, leading to deposition of fertilized eggs in the nest.

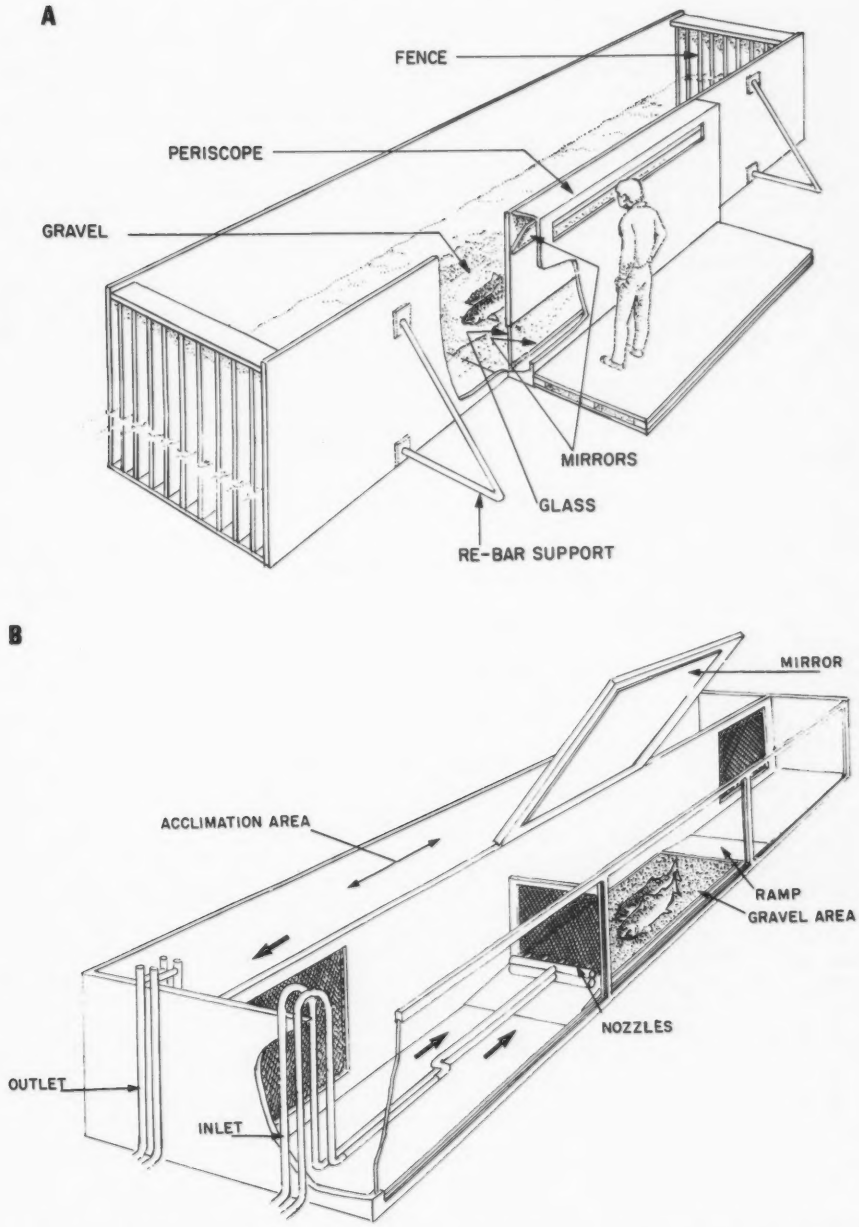

FIG. 1. A, Plywood flume used to study chum salmon (*Oncorhynchus keta*) spawning behavior. B, Indoor tank used to study rainbow trout (*Salmo gairdneri*) spawning behavior.

Three characteristic activities performed by the female during nest building are:

1) Digging — which consists of a series of strong body flexures while turned on one side. This digging creates a nest depression in the gravel in which eggs are eventually deposited (see Breder and Rosen [1966] for an extensive review).

2) Probing — described by Jones and Ball (1954) as a crouch which occurs during the latter stages of nest preparation. Jones (1959) describes this crouch as a behavior "in which the female, at first poised a few inches above the deepest portion of the bed, drops slowly and vertically downward with her anal fin erect, until it touches or slips between the larger stones at the bottom; on contact the downward movement is reversed and she returns to her original position." Each such movement is considered to be a probe in this paper; "crouch" is used to describe the position of the female at oviposition.

3) Covering — a different type of digging initiated immediately after oviposition which gradually blends into the digging of the next nest. It is characterized by an increased frequency (Sheridan 1960) and, except for the first digs, by an increased number of flexures per dig (McCart 1969).

During courtship display the male's position is usually to one side of the female with his head at the level of her caudal fin. Two characteristic activities shown by the male are:

1) Quivering — which is described in several species as a high frequency low amplitude undulation of the body.

2) Crossing over — which is a side to side movement by the male over the caudal peduncle region of the female (Hartman 1969).

Courtship display and nest building culminate in oviposition and release of sperm. Prior to oviposition, the female drops into the nest in a crouch position and the male moves into the nest beside her. The female opens her mouth immediately followed by the male, and at this point the reproductive products are released with both partners vibrating their caudal peduncle and anal fin. The eggs are then visible at the bottom of the nest and covering begins. The first few covering digs are very gentle and normally do not move any gravel. The currents created by digging lodge the eggs in the interstices of the larger rocks. Digging gradually becomes more vigorous as the female moves upstream.

Diel Activity

Figure 2 shows digging records for six pairs of chum salmon recorded on the hydrophone apparatus. Abrupt peaks in digging activity (marked by arrows) indicate covering of the nest immediately after spawning. Visual confirmation of spawning was obtained for those peaks occurring during the daylight period.

Chum salmon seem capable of spawning at any time during the day or night. Activity seems to be associated more closely with time to next spawning than with the light cycle, i.e. there is a repeated sequence of events leading to oviposition, and initiation of each sequence seems independent of the light cycle. The data appear to differ from those of Hartman (1969) who observed a diel pattern of digging in the Gerrard rainbow stock. However, the chum records were obtained for isolated pairs and effects of factors such as aggressive interactions and cover requirements were not considered.

Selection of Spawning Site

The chum salmon investigations fortuitously demonstrated one of the stimuli important in selection of a spawning site. As indicated earlier, the flume contained a gravelled area located between two areas covered by plywood intended to position the female in front of the periscope. However, the first pair initiated spawning activity at the extreme back end of the tank completely within the boundaries of the plywood sheet. Digging and quivering were observed for several hours and no obvious attempt was made to move upstream to the gravel. A few small rocks were present on the plywood near the female but as they were quite inconspicuous, it was felt that another cue must have determined the choice. Upwelling patterns associated with the end of the tank appeared to play some role in increasing the desirability of the location. Therefore, a number of rocks were placed at the back of the gravel area, creating a similar upwelling and accelerating flow. From that point all pairs initiated spawning near the back of the gravel area, immediately in front of the row of rocks. These observations are in agreement with those of White (1942), who also observed positioning of spawners near an accelerating flow.

As velocities were not manipulated in the chum salmon studies, it was impossible to distinguish a choice of upwelling, accelerating current from a choice of a faster velocity. However, data collected for rainbow trout did distinguish between these possibilities. Figure 3 shows the locations of nests constructed by rainbow trout under two velocity regimes with a mound of gravel (5 cm

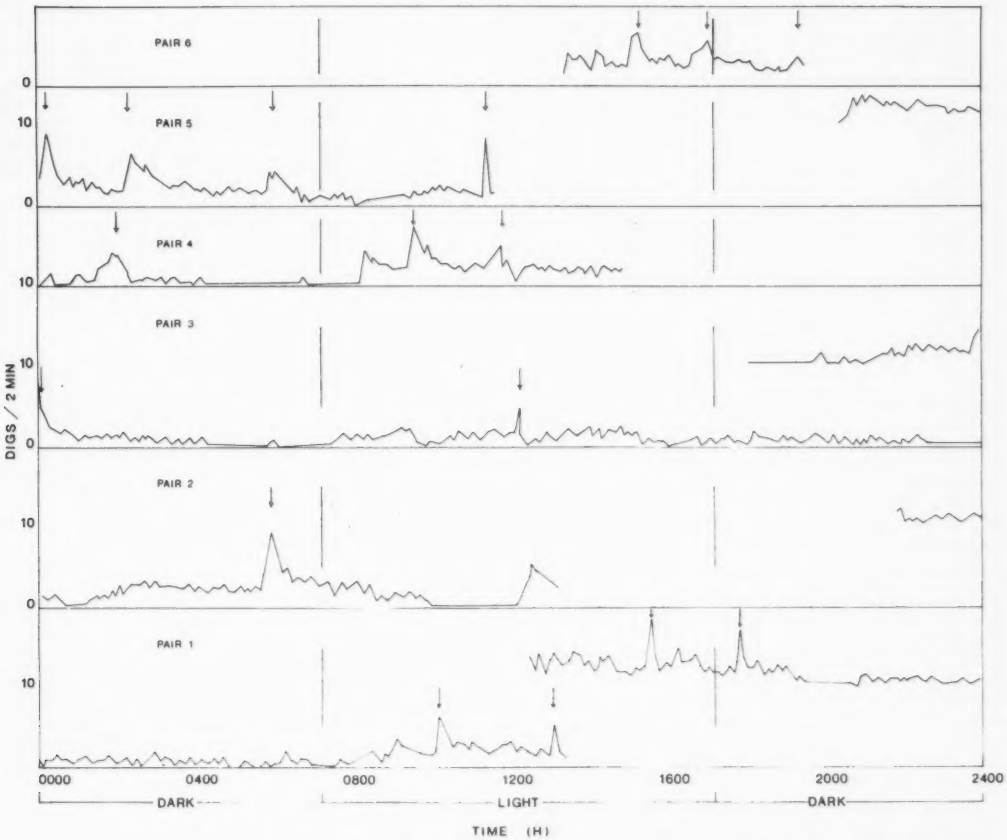

FIG. 2. Diel periodicity of chum salmon digging activity, arrows indicate time of egg deposition.

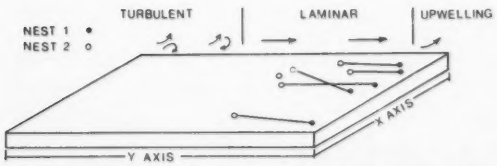

FIG. 3. Diagram showing nest locations, flow characteristics and orientation of the flume. Location of first nest indicated by black dot, location of second by white dot. The upwelling area is created by the 5 cm mound at the back of the tank.

high) located at the extreme back of the box. All initial nests were built immediately in the front of the mound indicating that velocity had little effect on the location of the first nest (Table 1). As a further test, the mound was moved upstream, with the result that the first nest of the next series was again located in front of the mound.

Finally, the fishes' behavior suggested that the accelerating–upwelling condition rather than velocity per se determined choice of location. Slow, passive, downstream, drifting with anal and pelvic fins touching the gravel was usually abruptly terminated when the fish encountered the accelerating current. On these occasions the fish would begin actively swimming upstream, or, quite often, a dig would be made. This was observed in low and high water velocities.

One additional complexity remains with regard to the selection of a site. Because of the hydrodynamic properties of the flume, the velocity

TABLE 1. Distances (centimeters) of first and second nest from mound for high (>18 cm/s) and low (<18 cm/s) velocities.

Nest no.	Distance (cm)	Velocity (cm/s)
1	15.0	27.4
2	56.5	15.8
1	10.4	15.2
2	61.3	18.3
1	20.8	12.8
2	82.4	35.3
1	21.3	28.3
2	65.5	17.7
1	16.3	12.8
2	59.0	35.3

range of the two flow conditions overlapped to some degree, i.e. areas in the high flow regime exhibited velocities identical to those exhibited by some other areas in the low flow regime and vice versa. Therefore, to eliminate the possibility that fish were in fact choosing the same velocity, irrespective of flow regime, it was necessary to consider the initial velocities at each nest site (Table 1). These data, again, suggest that absolute velocity is of secondary importance in the initial selection of a site.

CONSTRUCTION OF NEST

In this section, detailed aspects of nest building are considered for rainbow trout. Distance parameters required for describing the locations of each dig relative to the eventual location of the egg pocket are defined in Fig. 4. The average pattern of digging observed during nest construction is summarized in Fig. 5 which shows a small average movement of the female upstream during the construction of the nest, no significant left or right bias, and an apparent reduction in the estimated deviation of both X and Y as spawning approaches.[2]

Additional information is obtained from a study of individual digging records (Fig. 6). Here, localization of digging is not obscured by averaging and is clearly discerned as a rapid decrease in total distance from egg pocket (i.e. the combination of the X and Y components in

[2]These are in fact estimates of σ, not $\sigma_{\bar{x}}$, and therefore are not adjusted for sample size. This is necessary because a few nests may have required only, say, 180 digs and these numbers would not be included in the 200 digs prior to spawning estimates. Consequently, the statistics should be considered only as rough estimates of variability.

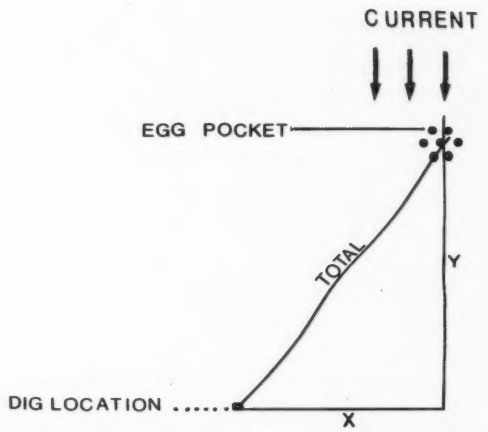

FIG. 4. Definition of variables used in describing dig position relative to the location of the egg pocket.

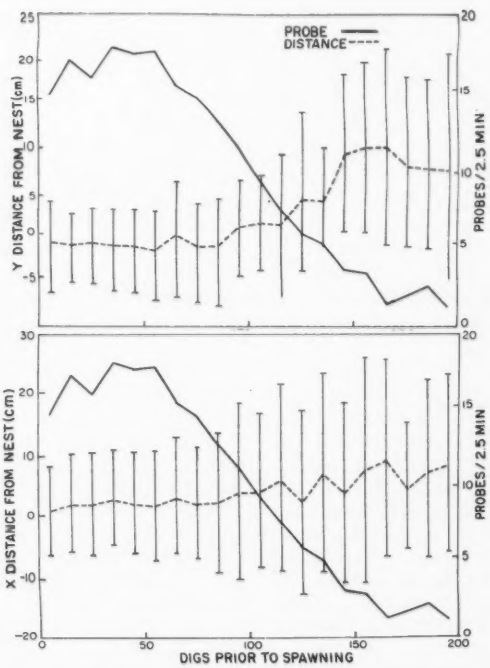

FIG. 5. Comparison of average upstream-downstream (Y) and left-right (X) distances from the egg pocket in relation to probing intensity. Vertical bars indicate two standard deviations.

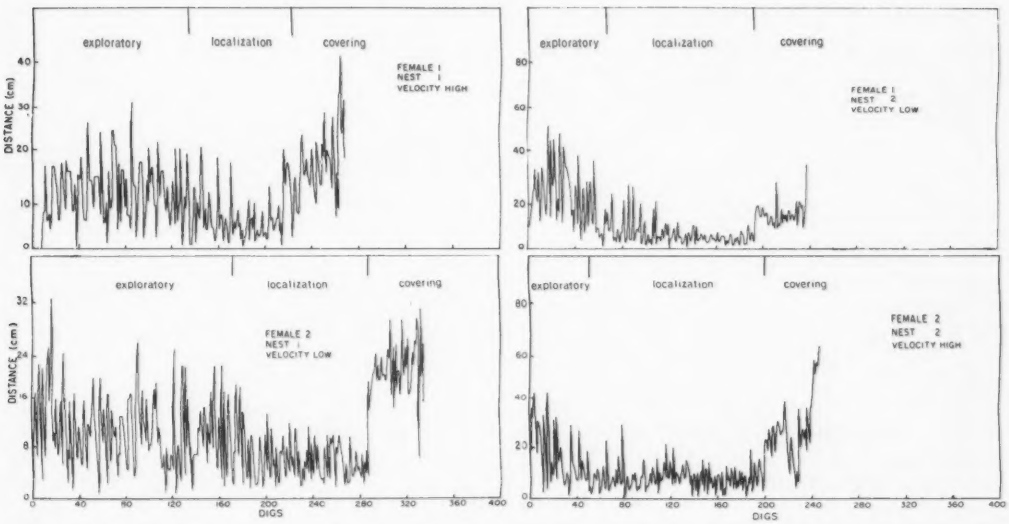

FIG. 6. Records of two females showing distance of dig from egg pocket under high and low velocity regimes.

Fig. 5) and a decrease in digging location variability. Covering is also clearly indicated by the rapid increase (upstream) in distance from nest. Further, it was apparent that increased probing activity and localized digging were associated with development of the nest depression, an observation consistent with female interaction with current patterns and/or nest shape.

EFFECTS OF ALTERED VELOCITIES

A two-way analysis of variance was performed on the number of digs completed prior to spawning, the effects being, (1) the first and second nest of a spawning sequence, and (2) conditions of high and low velocity. The average number of digs required was 267 (±32 S.E.) with no main effects significant. As the number of females in each category was small (3), no firm conclusions should be drawn. The data do suggest that number of digs required for nest 2 was slightly more than for nest 1 (279 vs. 255) and that more digging was required for nests constructed under conditions of high flow (275 vs. 255). Data obtained by estimating the distance traversed in each dig and reconstructing the nest from the film records indicated that there were no differences observed in angle of dig relative to current in the high and low flow condition, nor was any consistent difference apparent in size of area disturbed during construction of the nests.

FEMALE–MALE INTERACTION DURING SPAWNING

Frequencies per 2-min interval of male and female spawning behaviors as a function of time prior to spawning are provided for chum salmon and rainbow trout in Fig. 7.

The sequences are similar in that:

1) Probing is initiated between 100 and 130 min prior to spawning.

2) A small but consistent decrease in digging frequency is observed as spawning approaches.

3) The largest increase in frequency of male courtship behavior coincides with initiation of probing in the female.

Patterns differ in that male quivering is not as closely associated with probing activity in rainbow trout as in chum salmon, and that frequency of probing in chum salmon is approximately one half that observed for trout.

Behavioral activities occurred in a usually predictable order as oviposition approached. After completing a dig, the female drifted backwards so that her vent was over the egg pocket and initiated a probe. The male then approached the female from his position behind and to one side of her, quivering as he did so. At this point, the female usually completed the probe, i.e. lifted herself out of the crouch, and the male drifted backwards to his original location, often crossing over the back of the female (Fig. 8). A sequence which culminated in oviposition was similar ex-

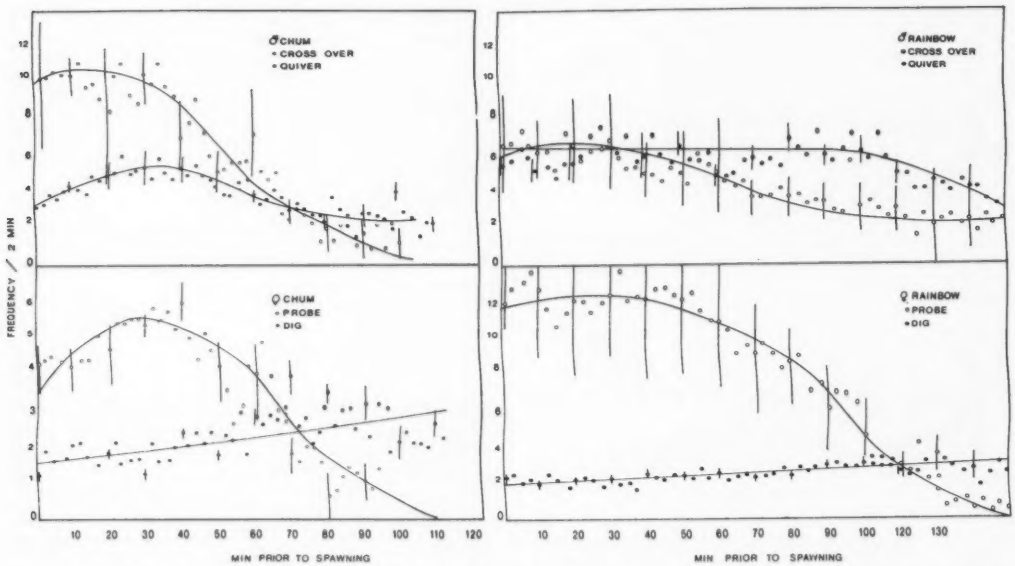

FIG. 7. Average frequency/2 minutes ±1 S.E. of male and female chum and rainbow trout spawning behaviors.

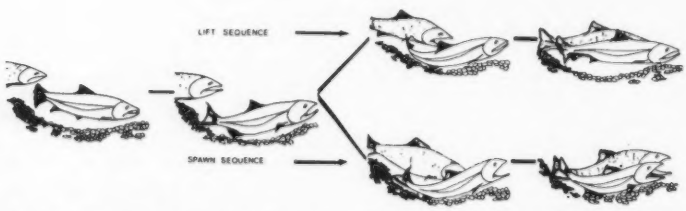

FIG. 8. Diagram comparing an early crouch sequence (lift) with one resulting in egg deposition (spawn).

cept that the female remained in the crouch, thereby allowing the male to assume a similar position beside her (Fig. 8).

FEMALE–NEST INTERACTIONS

The close temporal association of probing and quivering suggested that probing served as a signal to the male, indicating an increased readiness to spawn on the part of the female. It also seemed likely that probing was a means by which the female could monitor the structure of the nest. To support this hypothesis, it was desirable to show that, (1) some component of probing behavior change with change in nest structure, and (2) that digging behavior change in response to alterations in nest structure.

To satisfy the former condition, movie films obtained in other experiments containing probing and spawning sequences were studied. For each probe, the maximum angle of anterior–posterior body axis was measured relative to the gravel bed, e.g. if fish were monitoring depth of nest, the deeper the bed, the larger would be the angle recorded. These data are plotted for three separate spawnings (Fig. 9) but as the sequences were taken at random intervals (i.e. not every 5th, 10th, etc., probe was photographed), the data cannot show the gradual change in probing angle that would be expected if the female was con-

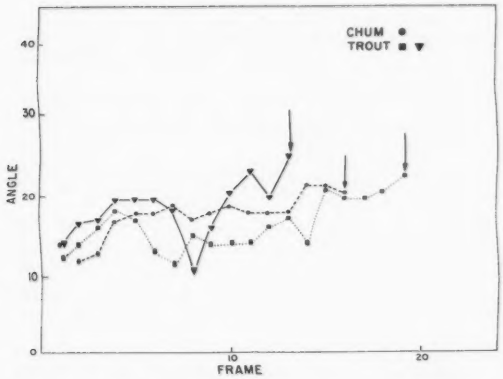

FIG. 9. Body position relative to gravel bed during probing in three spawnings of chum salmon and rainbow trout. Arrows indicate angle of probe at the time of spawning.

tinually monitoring nest shape. Nevertheless, the angle does increase as spawning approaches, indicating that probing could convey information to the female concerning the structure of the nest.

The second condition is more difficult to demonstrate. Without knowing exactly which nest cue(s) is responsible for eliciting oviposition, one cannot be certain of the degree to which any experimental manipulations have altered critical stimuli. Nevertheless, a depression was constructed in the shape of a nest, and a mature and unspawned female was directed to that area. The number of digs completed prior to spawning in this case (75), was substantially less than average for an initially level substrate (255), and the digging locations and variability were characteristic of the final stages of spawning (Fig. 10).

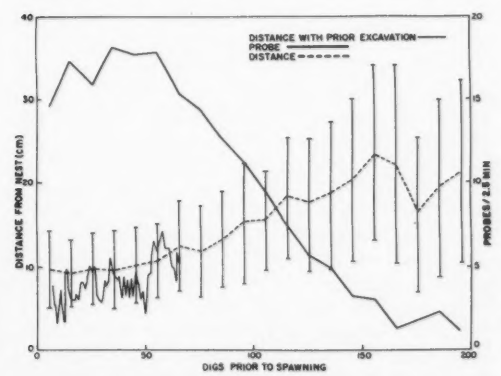

FIG. 10. Comparison of number and location of digs with and without prior excavation of the nest site. Vertical bars represent two standard deviations for average distance from egg pocket for all spawnings. Distances for the nest with prior excavating represent a running mean.

Discussion

In comparing these results with those obtained for other species (McCart 1969; Sheridan 1960; Leggett 1969; Jones and Ball 1954), it is clear that the basic components of spawning behavior are similar for most salmonids that spawn in streams. From the chum salmon work, it can be seen that these components are organized into repeated sets of activity, each set leading to oviposition, and each set being separated from the succeeding set by a variable period of time in which little serious spawning activity seemed to occur.

The female appeared to be the dominant partner, in the sense that most male activity (with the possible exception of early male quivering) occurred in response to, or at least immediately after, a particular female behavior. Most notable in this respect was the association between quivering and probing, which in the latter stages of spawning was a predictable action and reaction. Quantitative differences existing in probing and quivering between the species do not seem important and probably resulted from a slight difference in the probing technique of each species.

The ability of chum salmon and rainbow trout to detect upwelling currents, and the reduction in digging variability associated with development of the nest, suggest that the current pattern around the nest provides important locative information for the female. Further, probing seems to provide information concerning the shape and particularly the depth, of the nest. Together, these probably play a major role in determining time of oviposition.

Finally, velocity and gravel size would appear important only insofar as they influence the ease with which a suitable nest depression can be constructed. Early excavation of the nest is undoubtedly affected by velocity but as the nest takes shape and current patterns change, the major excavating force would appear to be the vortex created by the movement of the tail. The maximum size the female can lift is thus an important component of gravel composition inasmuch as the relative amount of this material in the substrate will determine the ultimate depth of the nest depression.

Acknowledgments

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Pectoral Fin and Opercular Movements of Atlantic Salmon (*Salmo salar*) Alevins

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PETERSON, R. H. 1975. Pectoral fin and opercular movements of Atlantic salmon (*Salmo salar*) alevins. J. Fish. Res. Board Can. 32: 643–647.

The pectoral fins of Atlantic salmon (*Salmo salar*) alevins change in shape and orientation as development proceeds. They flutter rhythmically at 100–150/min whereas opercula move infrequently in newly hatched alevins. Ablation of pectoral fins induces increased opercular movements. Rhythmic movements of the pectoral fins displace water from the posterior edge of the opercula to the area immediately behind the fins. The frequency of pectoral movements is influenced by changes in ambient PO_2. The pectoral movements are probably useful in generating water currents around the alevin in the gravel interstices.

PETERSON, R. H. 1975. Pectoral fin and opercular movements of Atlantic salmon (*Salmo salar*) alevins. J. Fish. Res. Board Can. 32: 643–647.

Les nageoires pectorales des alevins de saumon atlantique changent de forme et d'orientation à mesure que le développement avance. Elles battent rythmiquement au taux de 100–150 battements/min, alors que les opercules ne bougent que rarement chez les alevins nouvellement éclos. L'ablation des nageoires pectorales provoque une augmentation des mouvements operculaires. Les mouvements rythmiques des nageoires pectorales déplacent l'eau du bord postérieur des opercules à la région située juste derrière les nageoires. La fréquence des mouvements des pectorales est affectée par les changements du PO_2 ambiant. Les mouvements des pectorales servent probablement à engendrer des courants d'eau autour de l'alevin qui se trouve dans les interstices du gravier.

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ADULT Atlantic salmon (*Salmo salar*) excavate depressions (redds) in gravel where the eggs are deposited and buried. Hatched alevins remain in the gravel until the nutrient supply of the yolk sac is nearly exhausted, a period of about 6 mo.

Due to the relatively undeveloped state of the newly hatched alevin, and its curious mode of existence within gravel beds, one may expect physiological and behavioral adaptations. One of the most striking behavioral characteristics of the newly hatched alevin is the rapid, rhythmic, and synchronized flutter of the pectoral fin. White (1915) noted that the exposure of brook trout alevins to carbonated water resulted in an increased frequency of pectoral fin movement. Bishai (1962) observed that, although salmonid alevins, 1–4 wk after hatching, did not avoid water of low O_2 content (0.18–1.6 mg/liter), they did exhibit increased respiratory movement. Holeton (1971) commented that pectoral movements increased when the ambient PO_2 was lowered. This paper presents descriptive accounts of water currents generated by pectoral fin movement in Atlantic salmon (*Salmo salar*) alevins, and some quantitative data on the effects of varying O_2 tension on frequency of pectoral fin movements.

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Materials and Methods

Eyed salmon eggs of Maria River, Quebec, stock were hatched (density of 0.25/cm³) at temperatures of 3–13 C to provide newly hatched alevins available over a period of several months. Alevins less than 24 h after hatching were used unless indicated otherwise.

To study water movements (as revealed by dye tracers) generated by pectoral fin movements, the alevin was placed in a petri dish which in turn was suspended in a larger bowl filled with crushed ice. The temperature in the petri dish was 2–4 C. Usually the alevin was allowed to lie flat on the bottom of the dish, but occasionally it was induced to lie between two glass slides on the bottom of the dish so that the alevin's dorsal surface could be viewed. Photographs were taken of dye currents created by fin movements, and tracings were made of the photographs. Methylene blue solutions, applied with a Pasteur pipette, were used in the dye experiments.

Camera lucida drawings were made of alevins

preserved in formalin for comparison of their different stages of development.

The observation chamber used for counting fin-beat frequencies consisted of five wells (40 × 13 × 17 mm deep) milled into a block of plexiglass. Aerated water was introduced into one end of each well and ran out a hole at the opposite end. Water at a temperature of 9 ± 1 C ran to an aeration reservoir, then to the wells.

Alevins, 1–4 h after hatching, were placed in the wells 16 h before experimentation to allow the alevins to settle down. Recording and observation of fin-beat frequencies and opercular movements were performed at 125×.

Water of reduced O_2 tension was obtained by bubbling N_2 through it until the PO_2 was lowered to the desired concentration. Reservoirs containing water of lowered PO_2 were maintained at the temperature of the aerated water supply. O_2 tensions were measured with the electrode (Beckman Macroelectrode and Model 160 Analyzer) inserted into one of the wells. The electrode was calibrated with water at air saturation and with water exhaustively deoxygenated with N_2.

For ablation experiments the alevins were anesthetized in M.S. 222. The pectoral fins were cut off at the peduncle with iridectomy scissors. A portion of the tail fin was removed for sham operations. All alevins subjected to anesthetic and ablations recovered and survived for several weeks with no apparent ill effect.

Results

Shape and attitude of the pectoral fin — The shape and orientation of the pectoral fins change as the alevin develops (Fig. 1). Initially the pectoral fin is fan-shaped (Fig. 1A), and this shape is retained throughout most of the yolk-sac period (Fig. 1A, B, C). When the yolk sac is almost absorbed, the fin elongates, gradually assuming the triangular shape of the emergent fry (Fig. 1D, E). The orientation of the fin with respect to the body also changes. In the young alevin, the breadth of the fin is closely adducted to the side of the fish, whereas at completion of yolk-sac absorption, the fins are flattened dorso-ventrally with the thin inner edge next to the body.

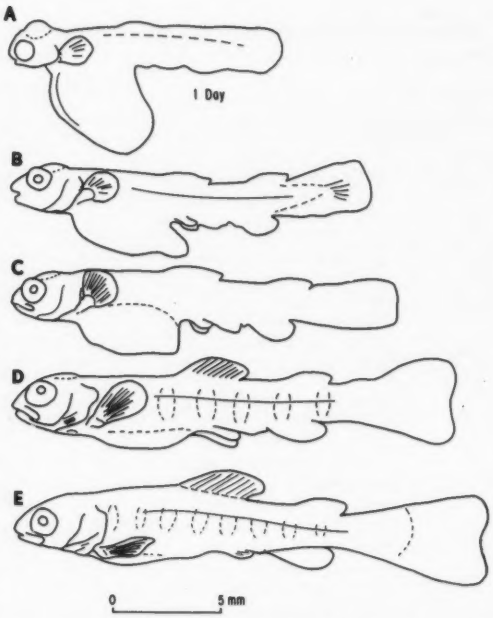

FIG. 1. Alevins at various stages of yolk-sac absorption from 1 day old (A) to near swim-up stage (E), showing changes in size and shape of the pectoral fin. Ages in the older alevins depend upon incubation temperature.

Water currents generated by pectoral movements — In the newly hatched alevin the pectoral fins maintain a constant rhythmic flutter of 100–150/min, adduction of one pectoral fin synchronized to the abduction of the other. This pectoral fin flutter is not a simple adduction–abduction movement of the fin as a whole. The anterior margin of the fin moves first in both adduction and abduction. In adduction, which is the power stroke of the cycle, a wave of movement travels down the fin from anterior to posterior thrusting a pulse of water posteriad as it moves. Occasionally both fins will stop for a few seconds, then resume at the same frequency.

Opercular movements are irregular with several minutes often passing with no opercular movement. The lower jaw moves regularly, although the amplitude is low with the mouth often not closing entirely. It is not known at present whether functional buccal valves are present in these alevins.

In six experiments the effects of fin movements on droplets of methylene blue solutions injected at various points near the alevin's body were observed. The pectoral flutter displaced water most effectively from the region just posterior of the operculum toward the area just posterior of the fins (Fig. 2). Dye placed just behind and above the head (Fig. 2A) was drawn in a thin line toward the fins, then was fanned back in a series of concentric shells, each stroke adding another layer. Conversely, a droplet of dye solution placed ventral to the gills (Fig. 2B) was not drawn toward the fins until it had diffused up to the

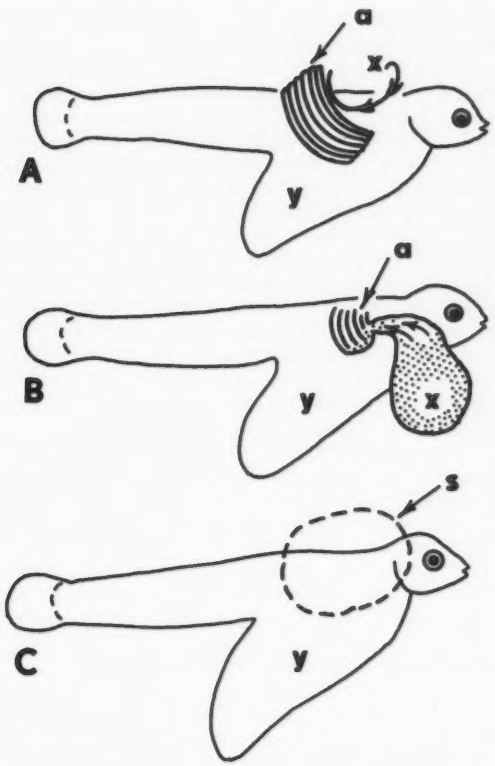

FIG. 2. Illustrations of water currents generated by the pectoral fins as indicated by dye movements. A, A spot of dye was placed near the posterior edge of the operculum (at "x"). The dye was thrust posteriorly as indicated by arrows, and was accumulated in a series of concentric layers (indicated by "a") in back of the fins. "y" = yolk sac. B, The dye was placed at "x"; however, the influence of the pectoral fins was small until the dye had diffused to the point indicated by the curved arrows. Stippled area indicates area of dye spreading by diffusion. C, The area enclosed by the dotted line indicates the "sphere of influence" of the pectoral fins, as indicated by the combined results of many dye presentations to a dozen alevins. Pectoral fins have not been included in the drawings.

area posterior to the gills. Pools of dye solution in front of the eyes, or at any point in back of or ventral to the sphere of influence of the fins were not affected.

When the dye was placed anterior to the alevin's mouth, some of it was incorporated into the respiratory current. All of the dye passing through the opercular cavity emerged through the most dorsal aspect of the opercular slit (Fig. 3A, B). From there the dye was drawn back by the pectoral flutter, then driven posterior to the fins in the pattern described above. If one of the pectoral fins is removed, the dye collects in a pool behind the operculum (Fig. 3C).

Fin-beat frequency and PO_2 of ambient water — The effects of lowering the PO_2 of the ambient water on pectoral fin frequency was studied with 10 alevins by visually counting the frequency. In general, lowering the O_2 tension was associated with a rise in fin-beat frequency (Fig. 4). The mean for air saturated water was 134/min, whereas at tensions of 60 mm Hg and lower, the mean rates were 160–180/min. The slope of the line of best fit relating fin-beat frequency to PO_2 is –0.329 fin beats/unit change in PO_2 (slope differed statistically from zero at $P < 0.001$).

Low O_2 tensions also increased opercular rates. In two older alevins (>72 h), the normal opercular rates were steady at 58–63/min and 40–55/min. Upon decreasing the O_2 tension to 60 mm Hg the rates increased to 66–71 and 63–79,

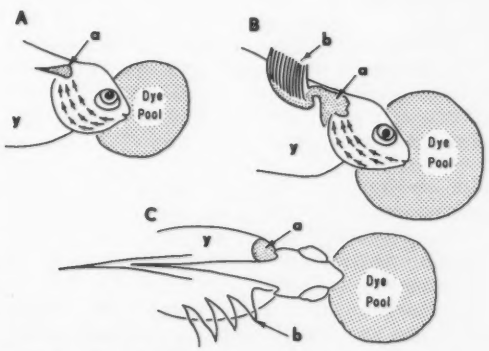

FIG. 3. The fate of water entering the mouth and passing through the opercular cavities is demonstrated by dye movements. A, Stippled area surrounding alevin's mouth represents the pool of dye. Arrows indicate movement of dye through the opercular cavity. The stippled shape (a) at the postero-dorsal corner of the operculum is dye that has passed through the opercular cavity; y is the yolk sac. B, Slightly later in the same sequence as A. Considerably more dye has passed through the opercular cavity and has been fanned back into the characteristic concentric layers (b). C, Dorsal view of alevin on which ablation of the left pectoral fin has been performed. On the left side, dye passing out of the opercular cavity collects in a pool (a). On the other side, with intact fin, the dye is swept back away from the operculum (b).

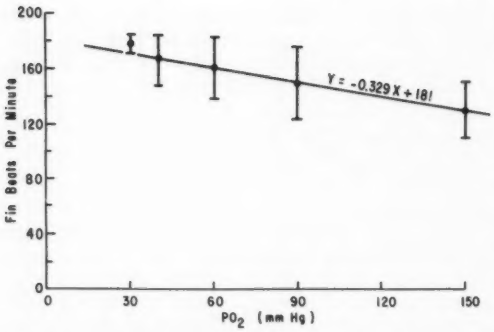

FIG. 4. The frequency of fin beat at various levels of ambient PO_2. The vertical bars show ±1 SD. The slope of –0.329 was significantly different from 0 at $P < 0.001$ ($t = 5.48$, $df = 54$).

respectively. In alevins less than 24 h old, the "normal" opercular rate was slightly under 6/min for five alevins (two measurements on each). For one alevin at 80 mm Hg the rate rose from 6/min to 23/min. For two others the rate rose to 15/min and 36/min, respectively, when the PO_2 was decreased to 30 mm Hg.

Pectoral fin ablation — Bilateral ablation of the pectoral fin was performed on six newly hatched alevins anesthetized in M.S. 222. Four other alevins of similar age were anesthetized and sham-operated by removing a part of the caudal fin. Two others were anesthetized only. All alevins survived and were apparently in excellent condition the next day. The mean opercular rate for alevins with bilaterally ablated pectoral fins was 58/min, whereas the mean opercular rate for intact alevins was only 22/min and that for the sham-operated alevins was only 20/min (Table 1). It was also obvious that the opercular movements of the finless alevins were much more forceful than of those with fins intact.

TABLE 1. Opercular and pectoral fin frequencies of Atlantic salmon (*Salmo salar*) alevins (counts per minute).

Intact		Sham-operated		Pectorals removed	
Gill	Fin	Gill	Fin	Gill	Fin
34	167	22	171	54	–
10	171	14	167	58	–
		25	182	47	–
		19	162	71	–
				56	–
				62	–
22	169	21	172	58	–

Discussion

The dye tracer experiments demonstrated that the pectoral fins were effective in propulsion of water from the posterior margins of the opercula to an area a few millimeters behind the fins. The fins are not effective in irrigating the yolk sac, except where it joins the body, nor do they irrigate most of the trunk. Whether or not their action facilitates passage of water through the opercular cavity is conjectural at this point. Normally the opercula of newly hatched alevins move infrequently. Under adverse conditions, such as decreased PO_2, frequency of opercular movements is increased (this work and Holeton [1971]).

The postulate of Stuart (1953) that the pectoral fin beats are used to maintain a silt-free area around the alevin may well be true. Low O_2 tension and silting go hand in hand in many circumstances. However, these experiments indicate that the fin beats would not effectively remove silt from the posterior part of the alevin or most of the yolk sac. It is probable that body movements would perform this function (the "ventilation swimming" of Bams [1969]).

The fact that ablation of the pectoral fins results in faster and more forceful movements of the opercula may be explained in two possible ways: firstly, neural centers controlling opercular movements may be inhibited by input from the center(s) controlling the fin beat. Ablation of the fins, through some sort of feedback, could release this inhibition. Secondly, lower O_2 tension in the opercular cavities, resulting from fin ablation, could stimulate opercular movements. This second possibility seems more likely as experimentally lowered O_2 tensions also initiated increased frequency of opercular movements.

It is appropriate here to relate the above discussions to conditions in the redds. Jones (1959) has stated that eggs are deposited in batches of a thousand or so in the gravel. P. F. Elson (personal communication) has observed pockets of 100 or so in redds that have been dug. Because it is impossible for newly hatched alevins to move to any great degree in gravel because of the large yolk sac (personal observations under seminatural conditions), one may assume that large numbers of alevins lie packed together in the redd for at least several hours after hatching. Creation of irrigation currents through narrow channels around the alevin in this mass is of great importance to survival and it is probable that pec-

toral fin movement contributes substantially to this end. Probably under adverse conditions both body movements and opercular movements also come into play. An observation of interest in this regard is that the pectoral fins are situated at an indentation where the yolk sac joins the body. This permits free movement of the fin even on the underside of an alevin lying on its side in a flat dish and seemingly would permit fin movement in crowded situations.

Hayes et al. (1951) determined an O_2 tension of 100 mm Hg to be critical for development of newly hatched alevins at 10 C. Thus, all the lowered O_2 tensions used in this paper would probably prevent normal development.

The changes in shape and orientation of the pectoral fins as the salmon alevin develops are adaptations to the changes that occur in the mode of existence of young salmon. The young alevin's pectorals are modified to generate water currents in a quite specific manner, probably associated with irrigation of alevins in the gravel.

Acknowledgments

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New Coccidia from the Spiny Dogfish Shark (*Squalus acanthias*) and Great Sculpin (*Myoxocephalus polyacanthocephalus*)

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FITZGERALD, P. R. 1975. New coccidia from the spiny dogfish shark (*Squalus acanthias*) and great sculpin (*Myoxocephalus polyacanthocephalus*). J. Fish. Res. Board Can. 32: 649–651.

New coccidia from the spiny dogfish shark, *Squalus acanthias*, and the great sculpin, *Myoxocephalus polyacanthocephalus*, from waters around the San Juan Islands, Puget Sound, Washington, are described and named. Thirty-one species of bony and two species of cartilaginous fishes were examined. Twenty-eight of 71 dogfish and one of four sculpins were infected with coccidia.

FITZGERALD, P. R. 1975. New coccidia from the spiny dogfish shark (*Squalus acanthias*) and great sculpin (*Myoxocephalus polyacanthocephalus*). J. Fish. Res. Board Can. 32: 649–651.

Nous décrivons et nommons de nouvelles coccidies trouvées chez l'aiguillat commun, *Squalus acanthias*, et le grand chaboisseau, *Myoxocephalus polyacanthocephalus*, des eaux du voisinage des îles San Juan, Puget Sound, Washington. Nous avons examiné 31 espèces de poissons osseux et deux de poissons cartilagineux. Vingt-huit des 71 aiguillats communs et un des quatre chaboisseaux étaient infestés de coccidies.

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RELATIVELY few species of coccidia have been described from marine fishes. Becker (1956) listed 40 species of *Eimeria* from marine and freshwater species of fish. Pellérdy (1974) listed only five valid species of *Eimeria* from the Chondrichthyes and 61 from the bony fishes. This paucity of information suggests that coccidiosis of marine fishes has not been well investigated. Under certain aquaculture conditions (Schäperclaus 1965) and some natural conditions (Kabata 1963) some species of *Eimeria* are associated with disease.

This report gives the results of a systematic search for coccidia in 33 species of fishes collected from the waters surrounding the San Juan Islands of Puget Sound, Washington, USA.

Materials and Methods

Cartilaginous and bony fishes were captured by trawl from approximately 46 m of water in East and West sounds, Orcas Island, San Juan Islands, Puget Sound, Washington. They were returned to the Friday Harbor Laboratories, University of Washington and transferred to holding tanks. A few small fish were captured by dipnet from tidepools around San Juan Island and a few salmon were taken by hook and line from waters near Sekiu, Washington. Thirty-three species of fish totaling 270 individuals were examined. Two new species of coccidia are described.

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Eimeria squali n.sp.

Description — Fig. 1 and 2. Freshly isolated oocysts were ovoid to ellipsoidal; 20–29 × 24–36 μm; brownish-lavender color under light microscopy; pitted with the bottoms of the pits light green or grey. Number of layers in the oocyst wall not determined. No micropyle visible. Sporont always spherical, located against the small end of the oocyst usually filling ⅓–½ the oocyst; diameter 16–28 μm, mean 19.9 μm. After sporulation four sporocysts present having a mean size of 5.9 × 19.6 μm, each with two sporozoites. Mean sporozoite measurements 2.2 × 13.6 μm laying slightly twisted in a "head-to-tail" arrangement; typically slender spindle-shaped. While still in the sporocyst each sporozoite typically with cross-striations weakly visible on the large end for about ⅓ its length, striations not visible outside of the sporocyst. Large spherical oocyst residuum 12.4 μm mean diameter. No organized sporocyst residuum, but several small round granules present in each sporocyst. Several fibers or mucous strands ex-

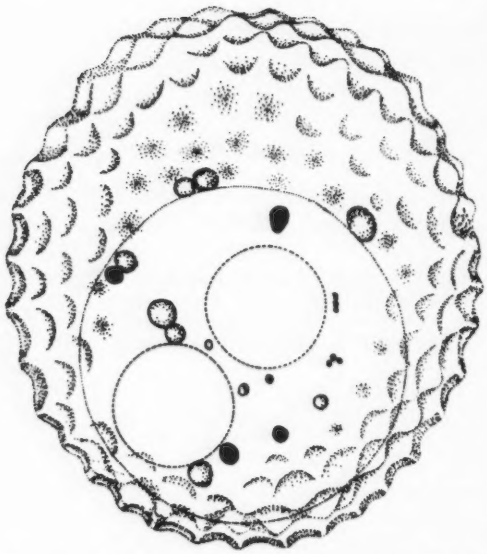

FIG. 1. Unsporulated oocyst of *Eimeria squali* n.sp. from the spiral valve of the spiny dogfish shark (*Squalus acanthias*).

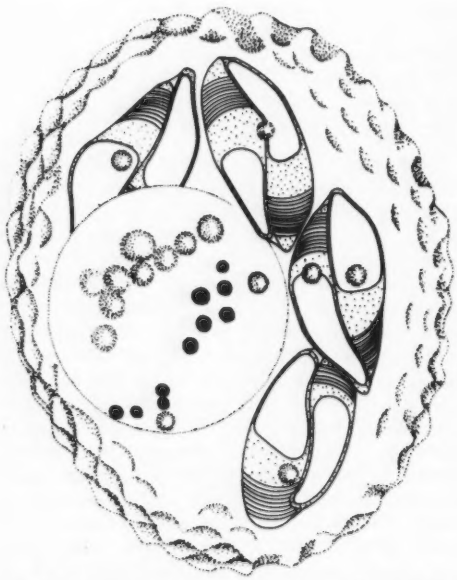

FIG. 2. Sporulated oocyst of *E. squali* n.sp. from the spiral valve of the spiny dogfish shark (*Squalus acanthias*) after sporulation in cold sea water.

tending from the pointed ends of the sporocysts when forced from the oocyst. This disappeared shortly after leaving the oocyst shell. Oocysts unsporulated when leaving the host.

Host — *Squalus acanthias* L., dogfish shark.

Location in host — Contents of the spiral valve and in mucosal scrapings of the spiral valves.

Habitat — Hosts taken from 46 m sea water from East and West sounds, Orcas Island, San Juan Islands, Puget Sound, Washington, USA.

Incidence of infection — Twenty-eight of 71 (39%) *Squalus acanthias* infected.

Comments — No coccidia were observed in any other organs. The number found varied from 200 to 3200 oocysts per gram of gut contents or tissue scrapings. Examination of 25 sharks held without food for several weeks had coccidia in scrapings from the mucosa of the empty spiral valves. There were no gross lesions in the spiral valves of any of the sharks and randomly selected histological sections contained no endogenous stages of the parasites.

Attempts to sporulate oocysts in 2.5% potassium dichromate failed. Unsporulated oocysts sporulated in cold, fresh sea water within 48 h after removal from the spiral valves.

Oocysts were recovered from mucus or spiral valve contents by sugar flotation. Oocysts tended to change shape from ovoid to spherical if left in contact with the sugar solution for two hours. Measurements were made on fresh material immediately after recovery.

Eimeria myoxocephali n.sp.

Description — Fig. 3. Oocysts spherical 34–40 μm with a mean diameter of 37.2 μm. Oocyst wall smooth, 2 μm thick, and double-layered. No micropyle. No oocyst residuum in sporulated oocysts taken from the intestine. Well-formed sporocyst wall not visible; a thin membrane tightly stretched over the sporozoites holding them together in a twisted position. A small, round sporocyst residuum usually present. Sporozoites elongated, sausage-shaped; mean measurement of 3.7 $\times$ 16.7 μm; a single refractile globule present.

Type host — *Myoxocephalus polyacanthocephalus* Pallas, great sculpin.

Location in host — Mucosal scraping of the upper intestine.

Habitat — Host taken from East and West

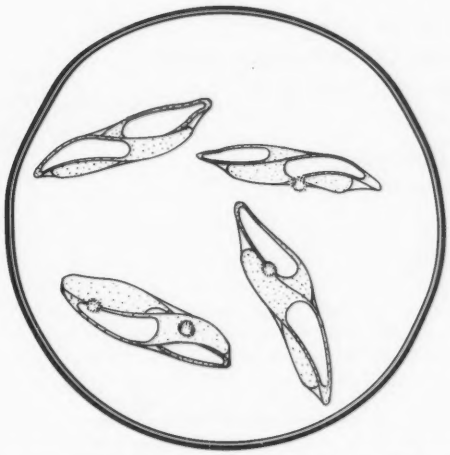

FIG. 3. Sporulated oocyst of *E. myoxocephali* n.sp. recovered from the intestine of the great sculpin (*Myoxocephalus polyacanthocephalus*).

sounds, Orcas Island, San Juan Islands, Puget Sound, Washington, USA.

Incidence of infection — One of four *Myoxocephalus polyacanthocephalus*, great sculpin.

Comments — The oocysts were sporulated when recovered from the intestine. The stomach was empty and only the lower intestine contained a small quantity of mucus and digested food. No other organs of this host were found to be infected with coccidia.

Discussion

Of the five recognized species of *Eimeria* described from cartilaginous fishes, none is similar to *E. squali*. *Eimeria southwelli* described from *Aetobatis narinari* (devilfish) by Halawani (1930), falls within the size range but is not pitted and otherwise has little resemblance.

None of the 17 species of *Eimeria* listed by Pellérdy (1974) in the order Perciformes falls within the size range of *E. myoxocephali*. *Eimeria variabilis* occurs in several other species in fish, including *Myoxocephalus bubalis*, a sculpin closely related to the great sculpin, but is less than half the diameter of *E. myoxocephali* and is unsporulated when passed. *Eimeria sardinae* described by Thelóhan (1890) from the testes of Clupeoid fishes (herring) is closest in appearance to *E. myoxocephali*. The size range is approximately the same, but the sporocysts are larger and sporozoites within the sporocysts are smaller than *E. myoxocephali*. Many herring were examined during the study but no coccidia were found in the testes or any other organ.

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Meristic Differences in Spawning Populations of American Shad, *Alosa sapidissima*: Evidence for Homing to Tributaries in the St. John River, New Brunswick

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CARSCADDEN, J. E., AND W. C. LEGGETT. 1975. Meristic differences in spawning populations of American shad, *Alosa sapidissima*: evidence for homing to tributaries in the St. John River, New Brunswick. J. Fish. Res. Board Can. 32: 653–660.

Univariate and multivariate analyses were performed on five meristic characters of American shad, *Alosa sapidissima*, spawning in the Miramichi, St. John, Connecticut, and Hudson rivers. In 71% of the comparisons, significant differences in mean numbers of meristic characters existed between river populations and between tributaries within the St. John River system. Comparisons of populations using multivariate techniques revealed that percentage of overlap of meristic characters was low (<1% in most comparisons). It is suggested that shad not only home to their natal river but also to their natal tributary. The results suggest that in shad, meristic counts are strongly influenced by a genetic component.

CARSCADDEN, J. E., AND W. C. LEGGETT. 1975. Meristic differences in spawning populations of American shad, *Alosa sapidissima*: evidence for homing to tributaries in the St. John River, New Brunswick. J. Fish. Res. Board Can. 32: 653–660.

Nous avons soumis à des analyses unies et multidimensionnelles cinq caractères numériques de l'alose savoureuse, *Alosa sapidissima*, frayant dans les rivières Miramichi, Saint-Jean, Connecticut et Hudson. Dans 71% des comparaisons, on trouve des différences significatives de valeurs moyennes des caractères numériques entre les populations des rivières et celles des tributaires dans le réseau de la rivière Saint-Jean. Les comparaisons de populations par des méthodes multidimensionnelles révèlent que le pourcentage de chevauchement des caractères numériques est faible (<1% dans la plupart des comparaisons). Nous suggérons que les aloses, non seulement retournent à leur rivière, mais aussi à leur tributaire natal. Ces résultats suggèrent que chez l'alose les comptages sont fortement influencés par un composant génétique.

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THE American shad, *Alosa sapidissima*, is reported to home to its natal river to spawn. This conclusion is based on observed differences in meristic (Fischler 1959; Hildebrand and Schroeder 1928; Hill 1959; Nichols 1966; Vladykov and Wallace 1938; Warfel and Olsen 1947) and life history (Carscadden and Leggett 1975; Leggett 1969; Walburg and Nichols 1967) characteristics between shad populations spawning in several Atlantic coast rivers and on tagging studies (Hollis 1948; W. C. Leggett in preparation; Talbot and Sykes 1958).

We recently reported (Carscadden and Leggett 1975) differences in life history characteristics among shad populations spawning in two tributaries and the main stem of the St. John River, N.B., and suggested that these differences may occur because of isolation resulting from shad homing to natal tributaries within the St. John River system. In this paper, we present the results of univariate and multivariate analyses of meristic characters of several Atlantic coast shad populations designed to further investigate the possibility of homing by shad to tributaries within the natal river.

Materials and Methods

The populations studied included the three St. John River stocks previously investigated for differences in population characteristics (Kennebecasis River, Washademoak Lake, and the main stem of the river at Mactaquac Dam; Carscadden and Leggett 1975) and populations spawning in the Miramichi, Connecticut, and Hudson rivers (Fig. 1).

Data Collection

Adult shad were collected from two tributaries of the St. John River, the Kennebecasis River, and

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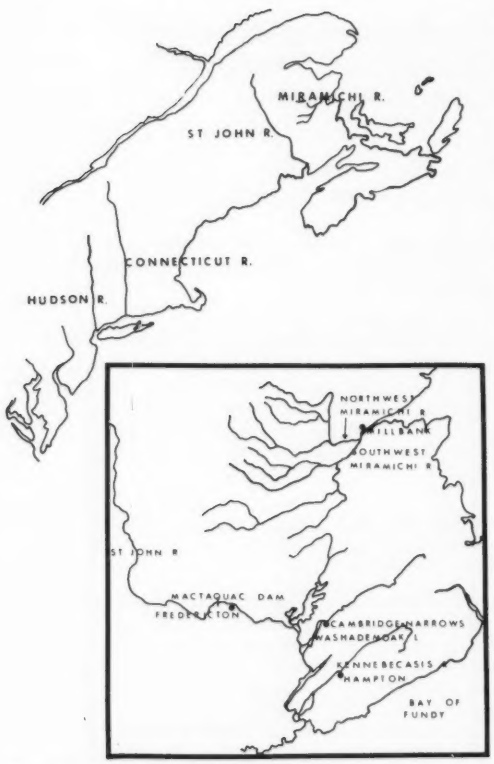

FIG. 1. Study areas on Atlantic coast and in St. John and Miramichi rivers.

Washademoak Lake, and from the main stem of the St. John River at Mactaquac Dam in 1972 and 1973. Sampling sites in the Kennebecasis River and Washademoak Lake were on or near the shad spawning grounds and considerable distance from the main stem of the St. John River. Shad arriving at Mactaquac Dam had to use the mechanical fish lift to reach the spawning area(s) upstream from the dam.

Kennebecasis River fish were taken with 11.4-cm, 12.7-cm, and 14.0-cm stretched mesh gillnets fished in the Hampton area approximately 48 km from the mouth of the St. John River. All Washademoak Lake samples were taken from commercial gaspereau trapnets operated in the Cambridge–Narrows area, approximately 88 km from the river mouth. Mactaquac Dam samples were obtained from the mechanical fish lift operated by the Department of the Environment. Mactaquac Dam is located approximately 140 km from the river mouth.

In 1972 and 1973, shad were collected in the Miramichi River from an adult salmon trapnet operated by Department of the Environment personnel at Millbank near its mouth. Approximately 24 km upstream from Millbank, the Miramichi River divides into two tributaries, the Southwest Miramichi River and the Northwest Miramichi River. In 1973, additional shad were collected from salmon trapnets operated by Department of the Environment personnel at various sites in both tributaries.

In the St. John and Miramichi rivers, 100 fish were selected at random from each sampling area annually. When the total annual sample was less than 100, all fish were kept. Number of rays in the dorsal, anal, and right pectoral fins and number of anterior and posterior scutes were counted for each fish (Hill 1959). Each fish was aged by the scale method (Cating 1953; Judy 1961).

Meristic data for the Hudson and Connecticut rivers were obtained from Hill (1959) who reported meristic counts on shad collected from the Hudson River in 1939 and 1940 and from the Connecticut River in 1945.

STATISTICAL ANALYSES

Initially all meristic data for each population were combined for use in interpopulation comparisons. The St. John River samples were then sorted into their respective year-classes and these were examined independently. Statistical comparisons were performed only on year-classes that contained more than 30 samples (Rothschild 1963). Year-classes meeting this criterion were Kennebecasis River, 1967 and 1968; Washademoak Lake and Mactaquac Dam, 1968 and 1969.

Means and standard errors were calculated for each meristic character in each population and where applicable, for each year-class. Significance of inter- and intrapopulation differences for each character were tested using the Student's *t*-test (Snedecor and Cochran 1967).

Discriminant function analysis was performed between all populations and where possible, between year-classes for each population. Assumptions of the discriminant function are discussed by Cooley and Lohnes (1971) and Bilton (1972). All computations were performed on an IBM 360 computer using an IBM discriminant function program (Systems/360 Scientific Subroutine Package, Version III). The Generalized Distance of Mahalanobis (Mahalanobis-D^2) was calculated by "multiplying the vector of the coefficients that constitute the discriminant function by the vector of differences between the means for the two groups" (Blackith and Reyment 1971). The square root, D, of the Mahalanobis-D^2 has been employed as a measure of distance between the populations compared (Mais 1972; Royce 1964). Mahalanobis-D^2 was corrected for uneven sample size and converted to percent overlap (Royce 1964). Percent overlap is an estimator of the amount of overlap in meristic characters between populations.

Results

Interpopulation differences in mean values of the five characters examined were small (Table 1).

TABLE 1. Means, standard errors, and ranges for meristic characters of American shad (*Alosa sapidissima*) populations composed of many year-classes from study areas on Atlantic coast.

Meristic character	No. fish	Mean	SE of mean	Range
Kennebecasis R.				
Dorsal fin rays	191	18.1	0.05	16–20
Anal fin rays	191	22.1	0.07	20–25
Pectoral fin rays	191	16.2	0.05	14–18
Anterior scutes	191	22.3	0.05	20–24
Posterior scutes	191	15.6	0.06	14–17
Washademoak L.				
Dorsal fin rays	219	18.1	0.05	16–20
Anal fin rays	219	21.9	0.06	19–25
Pectoral fin rays	219	16.5	0.04	15–18
Anterior scutes	219	22.2	0.04	20–24
Posterior scutes	219	15.5	0.05	13–17
Mactaquac Dam				
Dorsal fin rays	178	17.8	0.06	15–20
Anal fin rays	178	21.4	0.07	19–24
Pectoral fin rays	178	15.9	0.04	15–17
Anterior scutes	178	22.0	0.06	19–24
Posterior scutes	178	15.5	0.06	13–17
Southwest Miramichi R.				
Dorsal fin rays	77	17.8	0.09	16–19
Anal fin rays	77	21.3	0.10	19–23
Pectoral fin rays	77	15.9	0.07	15–17
Anterior scutes	77	22.5	0.08	21–24
Posterior scutes	77	16.2	0.08	15–18
Northwest Miramichi R.				
Dorsal fin rays	63	17.6	0.09	16–19
Anal fin rays	63	21.4	0.12	20–23
Pectoral fin rays	63	15.9	0.08	15–17
Anterior scutes	63	22.4	0.07	21–24
Posterior scutes	63	15.8	0.10	14–18
Millbank				
Dorsal fin rays	159	17.6	0.06	16–19
Anal fin rays	159	21.3	0.07	19–24
Pectoral fin rays	159	15.9	0.05	14–18
Anterior scutes	159	22.4	0.06	20–25
Posterior scutes	159	16.1	0.06	14–18
Hudson R.				
Dorsal fin rays	104	17.9	0.08	14–20
Anal fin rays	104	21.6	0.09	19–25
Pectoral fin rays	104	15.8	0.07	14–17
Anterior scutes	104	22.0	0.06	20–23
Posterior scutes	104	.15.3	0.08	13–18
Connecticut R.				
Dorsal fin rays	91	17.2	0.09	15–19
Anal fin rays	91	21.2	0.10	19–23
Pectoral fin rays	91	14.7	0.09	13–16
Anterior scutes	91	21.6	0.07	20–23
Posterior scutes	91	14.9	0.09	13–17

There were no obvious latitudinal trends in the mean counts. The Connecticut River samples exhibited lower mean meristic values than any of the other populations.

Although the interpopulation differences were small, significant differences ($P < 0.05$) existed in 99 of 140 (71%) possible comparisons (Table 2). Again no obvious trends occurred in the frequency of significant differences per comparison either with latitude or with increasing distance between home rivers. For example, the Hudson River and Mactaquac Dam populations were more similar in the characters investigated than the Hudson River and Connecticut River populations and differed no more than the samples from the Northwest and Southwest Miramichi rivers. Posterior scutes differed significantly in 23 of 28 comparisons, followed by dorsal fin rays and anterior scutes (21 of 28), pectoral fin rays (18 of 28), and anal fin rays (16 of 28).

The results of the multivariate analyses (Table 2) indicated that in spite of the small differences between individual characters, the overlap between populations was very low (<1% in 23 of 28 comparisons). There was no apparent trend in the magnitude of D^2 with distance between rivers. In this respect, the Mactaquac Dam and Hudson River populations differed in only one character and showed the highest percent overlap of all comparisons.

Between year-class comparisons of the mean meristic counts (Tables 3–5) of the Kennebecasis River, Washademoak Lake, and Mactaquac Dam populations revealed no significant differences. In contrast, several significant differences occurred when interpopulation comparisons of single year-classes were performed. In the 1968 comparisons, Kennebecasis River and Washademoak Lake shad differed in mean numbers of anal fin rays ($P < 0.01$); Kennebecasis River and Mactaquac Dam shad exhibited significant differences in mean numbers of anal and pectoral fin rays ($P < 0.01$); and shad from Washademoak Lake and Mactaquac Dam differed in mean numbers of pectoral fin rays ($P < 0.01$). Washademoak Lake and Mactaquac Dam shad from the 1969 year-class differed in mean numbers of anal fin rays ($P < 0.05$), pectoral fin rays, and anterior scutes ($P < 0.01$).

The between year-class comparisons within single populations revealed larger overlap percentages (Table 6) than within year-class comparisons between populations (Table 7). There was a general agreement between the multivariate and univariate comparisons. Less overlap occurred between populations that also exhibited greater

TABLE 2. Significant differences[a] between means of meristic characters[b], corrected Mahalanobis-D^2, and percent overlap for comparisons of Atlantic coast shad populations composed of more than one year-class.

	Meristic characters	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
		Connecticut R.					*Hudson R.*					*Kennebecasis R.*					*Washademoak L.*					*Mactaquac Dam*					*Millbank*					*Southwest Miramichi R.*					*Northwest Miramichi R.*			
Connecticut R.							**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**		**	**	**	**		**	**	**	*		**	**	**
	D^2	% overlap																																		
Hudson R.	153.42065	<1						*	**	**	**	*	**	**	**	*				*		**	**		**	**		*		**	**	*			**	**
			D^2	% overlap																																
Kennebecasis R.	543.22601	<1	64.46390	<1								*	**				**	**	**			**	**	**	**	**	**	**	**	*	**	**	**	**			
					D^2	%overlap																															
Washademoak L.	664.34043	<1	102.01306	<1	24.13962	1.4																**	**	**	**		**	**	**	**	**	**	**	**	**	**	*	**
							D^2	% overlap																														
Mactaquac Dam	272.09641	<1	11.61597	8.9	91.88819	<1	142.28361	<1																	**			**	**			**	**			**	**
									D^2	% overlap																												
Millbank	369.65873	<1	101.81591	<1	151.21577	<1	228.18148	<1	85.78123	<1																	•									**
											D^2	% overlap																										
Southwest Miramichi R.	322.03491	<1	79.71118	<1	91.60866	<1	127.89642	<1	70.55942	<1	6.10818	21.5																								**
													D^2	% overlap																						
Northwest Miramichi R.	217.73230	<1	43.74361	<1	55.84948	<1	86.38065	<1	29.81653	<1	6.13495	21.5	9.79750	11.6																						

[a] $*P < 0.05$; $**P < 0.01$.

[b] 1, Dorsal fin rays; 2, anal fin rays; 3, pectoral fin rays; 4, anterior scutes; 5, posterior scutes.

TABLE 3. Means, standard errors, and ranges for meristic characters for shad from Kennebecasis River, 1967 and 1968 year-class.

Meristic character	No. fish	Mean	SE	Range
Kennebecasis R., 1967 year-class				
Dorsal fin rays	53	18.0	0.10	16–19
Anal fin rays	53	22.0	0.13	20–24
Pectoral fin rays	53	16.2	0.10	14–18
Anterior scutes	53	22.2	0.10	20–24
Posterior scutes	53	15.7	0.10	14–17
Kennebecasis R., 1968 year-class				
Dorsal fin rays	38	18.1	0.10	17–19
Anal fin rays	38	22.3	0.14	20–24
Pectoral fin rays	38	16.3	0.08	15–17
Anterior scutes	38	22.3	0.10	21–24
Posterior scutes	38	15.4	0.12	14–17

TABLE 4. Means, standard errors, and ranges for meristic characters for shad from Washademoak L., 1968 and 1969 year-class.

Meristic character	No. fish	Mean	SE	Range
Washademoak L., 1968 year-class				
Dorsal fin rays	83	18.0	0.07	17–19
Anal fin rays	83	21.9	0.09	19–24
Pectoral fin rays	83	16.5	0.06	15–18
Anterior scutes	83	22.1	0.07	21–24
Posterior scutes	83	15.5	0.07	13–17
Washademoak L., 1969 year-class				
Dorsal fin rays	65	18.1	0.08	16–19
Anal fin rays	65	21.8	0.12	20–24
Pectoral fin rays	65	16.4	0.07	15–18
Anterior scutes	65	22.3	0.08	21–24
Posterior scutes	65	15.6	0.09	14–17

numbers of significant differences in the univariate comparisons.

A general trend towards decreasing mean numbers of characters with increasing distance of the tributary from the mouth of the St. John River was noted.

Discussion

Significant interpopulation differences and low overlap values in comparisons involving many year-classes were the rule, strongly suggesting that homing to tributaries within the natal river was occurring. Although the presence of several year-classes of unequal size could be a disadvantage in this analysis, the consistent mean meristic counts from the St. John River populations suggest that the inclusion of several year-classes in a sample is not a serious drawback. All comparisons involving more than one year-class exhibited significant differences. This is evident both in comparisons involving populations widely separated such as the St. John River–Connecticut River comparisons and in comparisons of populations in close proximity, such as those of the St. John River tributaries. Furthermore, the fact that between-tributary overlap values were no higher than between-river values suggests that homing to a tributary is as accurate as homing to the river itself.

Some exceptions to this trend of low overlap values from the multivariate analysis did occur. The largest overlap values occurred between the Hudson River–Mactaquac Dam, Southwest Miramichi River–Millbank, Northwest Miramichi River–Millbank, and Southwest Miramichi River–Northwest Miramichi River comparisons. We have no explanation for the similarity of the Hudson River–Mactaquac Dam meristic values. The high overlap values obtained when comparing the Northwest Miramichi River–Millbank and Southwest Miramichi River–Millbank samples were expected because the Millbank sample was composed of shad from both tributary populations. The relatively high overlap (11.6%) between the Northwest and Southwest Miramichi River samples is a significant deviation from the general pattern presented. It could result from mixing of members of several subpopulations in these samples. No detailed knowledge of the spawning of shad in the Miramichi River system is available to assess the possibility.

TABLE 5. Means, standard errors, and ranges for meristic characters for shad from Mactaquac Dam, 1968 and 1969 year-class.

Meristic character	No. fish	Mean	SE	Range
Mactaquac Dam, 1968 year-class				
Dorsal fin rays	45	17.8	0.13	16–19
Anal fin rays	45	21.6	0.14	20–24
Pectoral fin rays	45	15.9	0.09	15–17
Anterior scutes	45	22.0	0.12	20–24
Posterior scutes	45	15.5	0.11	14–17
Mactaquac Dam, 1969 year-class				
Dorsal fin rays	67	18.0	0.08	16–19
Anal fin rays	67	21.4	0.11	20–24
Pectoral fin rays	67	15.8	0.06	15–17
Anterior scutes	67	22.0	0.10	20–24
Posterior scutes	67	15.6	0.10	13–17

TABLE 6. Corrected Mahalanobis-D^2 and percent overlap for between year comparisons within American shad populations from the St. John River.

Population	Comparison	Corrected Mahalanobis-D^2	% overlap
Kennebecasis R.	1967–68 year-classes	4.85613	26.3
Washademoak L.	1968–69 year-classes	5.88844	22.6
Mactaquac Dam	1968–69 year-classes	6.76100	19.4

TABLE 7. Corrected Mahalanobis-D^2 and percent overlap for within-year comparisons between American shad populations from the St. John River.

Comparison	Year-class	Corrected Mahalanobis-D^2	% overlap
Kennebecasis R.–Washademoak L.	1968	11.66691	8.7
Kennebecasis R.–Mactaquac Dam	1968	20.46300	2.4
Wasahdemoak L.–Mactaquac Dam	1968	29.18876	0.7
Washademoak L.–Mactaquac Dam	1969	49.44464	0.05

The results of the comparisons of St. John River populations composed of one year-class also support our theory that shad are homing to their natal tributaries. The meristic counts within any one population were constant from year to year and the corresponding percent overlap values were relatively high. In contrast, within year-class comparisons between populations revealed numerous significant differences in meristic characters. Multivariate analysis between these same populations indicated the percentage overlap in characters was low.

The overlap percentages calculated from the Mahalanobis-D^2 values were considerably lower than those reported by Mais (1972) for sardines and Royce (1964) for tuna. These strictly pelagic marine species may intermingle to a greater extent during their reproductive phase, thereby producing greater overlap. Homing by shad to natal rivers and to natal tributaries would result in the reduced overlap observed. The characteristics measured may also contribute to these smaller overlap values. We measured discrete variables, whereas Mais and Royce measured continuous variables (morphometrics). Mayr (1969) noted that "meristic characters, such as number of spines, scales or fin-rays permit greater accuracy than do measurements." The differences between the intrapopulation and interpopulation overlap percentages were sufficiently large to strongly suggest that differences between the shad populations in the St. John River tributaries are real. Mais (1972) noted that low overlap values provide "good evidence of little or no intermingling."

The determination of numbers of meristic characters is known to be influenced both by the environment and by the genetic makeup of the organism (see Barlow 1961; Garside 1966; Taning 1952 for discussions and literature reviews). Water temperature is one important environmental variable influencing meristic counts. Although shad spawn at different times of the year at different latitudes (Leggett 1969; Walburg and Nichols 1967) and arrive on the spawning grounds in the tributaries of the St. John River at slightly different times (unpublished data), it is known that the timing of the peak of shad spawning migrations in all Atlantic coast populations is closely correlated with a narrow range of water temperatures (13–18 C). This is a species-specific

response and it ensures that the eggs and young develop under optimum thermal conditions (Leggett and Whitney 1972). However, the optimum temperatures for egg and larval development in shad range between 15.5 and 26.5 C (Bradford et al. 1968; Leim 1924; Marcy 1969). Therefore, it is possible that subtle localized temperature shifts during critical stages of development could affect meristic counts. Other environmental factors such as oxygen (Garside 1966; Taning 1952), light (Lindsey 1958), and others (see Barlow 1961) also influence meristic counts. The effects of these influences are clearly complex and are further complicated by the fact that meristic characters in most fish are genetically controlled by a multifactor system (Barlow 1961).

The consistent low variability in mean meristic counts between year-classes suggests that genetic regulation of meristic characters is significant in shad. The cline of decreasing meristic counts with increasing distance of the St. John River tributaries from the mouth of the river may also have resulted from genetic factors. A stable environment within each tributary from year to year could yield similar results. However, very small environmental changes occurring at critical developmental stages can significantly alter meristic counts (Taning 1952). It seems unlikely that such a highly stable environment exists between years in the variable climatic conditions of the spring season at this latitude. No measurements of environmental variables are available for the year-classes compared in this study. Therefore, the relative importance of environment and genotype in determining the trends we have reported remain unknown.

In summary, the results reported here support both the previous conclusions that shad home to a river system and our theory (Carscadden and Leggett 1975) that shad home to natal tributaries within a specific river system. In addition, our results suggest that in shad, meristic counts are strongly influenced by a genetic component.

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Effect of DDT and M.S. 222 on Learning a Simple Conditioned Response in Rainbow Trout (*Salmo gairdneri*)

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MCNICHOLL, P. G., AND W. C. MACKAY. 1975. Effect of DDT and M.S. 222 on learning a simple conditioned response in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 661–665.

Rainbow trout (*Salmo gairdneri*) were force fed, with and without M.S. 222 anesthesia, pellets containing DDT equivalent to 0, 10, and 100% of the 96 h-LD50 dose of 0.03 mg DDT/g body weight. DDT at the LD50 dose significantly increased learning rate 48 and 72 h after treatment. Although the 10% LD50 group did not learn significantly faster than controls, their slightly improved learning rate indicated a dose-dependent response curve. M.S. 222 did not affect learning rate of a simple conditioned response whether or not the anesthetic was combined with DDT.

MCNICHOLL, P. G., AND W. C. MACKAY. 1975. Effect of DDT and M.S. 222 on learning a simple conditioned response in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 661–665.

Des truites arc-en-ciel (*Salmo gairdneri*) anesthésiées et non anesthésiées au M.S. 222 ont été gavées de boulettes cõntenant du DDT en concentrations équivalentes à 0, 10 et 100% de la DL50 après 96 h de 0.03 mg de DDT/g de poids corporel. Le DDT à la concentration de la DL50 augmente significativement le taux d'apprentissage 48 et 72 h après traitement. Alors que l'apprentissage du groupe de 10% de la DL50 n'est pas significativement plus rapide que celui des témoins, un taux légèrement supérieur indique une courbe de réponse dépendante de la dose. M.S. 222 n'affecte pas le taux d'apprentissage d'une simple réponse conditionnée, que l'anesthésique soit combiné ou non avec le DDT.

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DDT (2, 2-bis (*p*-chlorophenyl)-1, 1, 1-trichlorethane) is widely known for its general action on the nervous system (e.g. O'Brien 1967) as well as specific effects on the brain (e.g. Dési et al. 1966; Woolley and Barron 1968; Aubin and Johansen 1969). Its effects on learning are well documented in rats (Khairy 1959; Thompson and Lilja 1964), mice (Al-Hachim and Fink 1968; Sobotka 1971; Craig 1972), and fish (Anderson and Peterson 1969; Anderson and Prins 1970; Hatfield 1970; Jackson et al. 1970; Anderson 1971; Hatfield and Johansen 1972). In all of the studies on fish cited above, DDT was administered in the water. However, the food chain is the major source of DDT in natural waters (Macek and Korn 1970). Thus, the present study examined the effect on learning rate of a single oral dose of DDT given to rainbow trout (*Salmo gairdneri*).

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M.S. 222 (ethyl m-aminobenzoate methanesulphonate) is a widely used fish anesthetic (Wedemeyer 1970). Because of its action on the central nervous system (McFarland 1960) it may act synergistically with DDT. It has also been shown to affect some behavioral responses (Goddard et al. 1974) and central autonomic functions (Houston et al. 1971) in fish. As M.S. 222 was used to anesthetize fish to facilitate force feeding in this study, a second part of the study compared learning rate of M.S. 222 and non M.S. 222-treated fish at all DDT doses used.

Materials and Methods

Fingerling rainbow trout were obtained from the Provincial Trout Rearing Station, Raven, Alberta. They were held at 15 ± 1 C, 12L:12D photoperiod for at least 2 wk in fiberglass tanks that received a continuous flow of aerated, filtered, dechlorinated Edmonton tap water. Four days prior to use in a given experiment, fish were individually marked with numbered fry tags placed in the musculature just anterior to the caudal fin.

TABLE 1. Mean weights, trials, and errors to criterion of each of the 12 groups.

Group[a]	Mean weight (g)	Weight range (g)	Mean trials to criterion ± 1 SE	Mean errors to criterion ± 1 SE
C-M.S.-48	23.0	13.6–31.7	30.1±3.0	8.8±1.0
C-M.S.-72	23.0	15.2–30.6	34.1±2.9	9.0±0.8
C-non-48	23.0	12.0–30.3	33.0±2.6	10.1±0.9
C-non-72	23.2	15.8–37.1	27.6±1.3	8.3±0.6
0.1-M.S.-48	23.1	14.5–38.1	29.6±2.6	8.3±1.1
0.1-M.S.-72	23.4	16.2–36.9	26.1±1.7	7.5±0.7
0.1-non-48	23.1	10.5–32.0	30.1±1.8	9.3±0.5
0.1-non-72	23.1	12.6–37.3	27.6±3.4	8.1±1.5
1.0-M.S.-48	23.2	13.2–41.3	23.0±1.6	5.8±0.6
1.0-M.S.-72	22.9	13.8–31.5	25.0±1.9	7.6±0.6
1.0-non-48	23.2	15.5–30.8	23.6±2.2	6.6±1.2
1.0-non-72	23.1	14.7–31.8	23.0±1.0	5.4±0.6

[a]The groups are named as follows: the first of the three symbols refers to the dose (C[control], 0.1 or 1.0 tu of DDT), the second to the use of the anesthetic (M.S. = M.S. 222 use. non = no M.S. 222) and the third to the time of testing after DDT administration (48 or 72 h). N = 8 for all groups.

Prior to the conditioning experiments, the 96 h-LD50 dose was determined (i.e. the dose required to kill half the fish in 96 h). To determine whether delayed mortality occurred, some fish surviving beyond 96 h were kept for at least a week. Doses of DDT used in the conditioning experiments were the 96 h-LD50 level, or 1.0 tu (toxic units, as in Sprague 1970), 10% of the 96 h-LD50 or 0.1 tu, and 0 tu (hereafter referred to as controls).

One day prior to DDT administration, fish were individually weighed (see Table 1 for group mean weights) and assigned to one of the three doses indicated above. Groups of fish receiving each dose were further subdivided into those that were anesthetized with M.S. 222 while being given DDT and those that were not. Each of these was again divided into those tested 48 or 72 h after DDT administration. Thus a total of 12 groups was formed (Table 1). Eight fish were tested in each group.

A food pellet (Silver Cup Trout Pellet) was prepared for each test fish so that all fish in a group received the same quantity of DDT per gram body weight. The DDT (analytical grade *p, p'*-DDT, 99+% pure) was dissolved in petrolum ether and the appropriate volume was drawn into a 50 μl syringe. The solution was dropped slowly onto a food pellet so that as the solvent evaporated, the DDT coated and partially penetrated the pellet. When fish were force-fed such pellets, any DDT that rubbed off would tend to adhere to and be absorbed by the fish, with its high fat content, rather than be lost in the water where it is virtually insoluble (O'Brien 1967). As the volume of DDT solution was generally less than 50 μl, controls received 50 μl of solvent on a food pellet. The solvent was allowed to evaporate overnight from the food pellets. It is unlikely there was much evaporative loss of DDT as it has exceedingly low vapor pressure (O'Brien 1967).

The DDT was administered by force-feeding each fish with the appropriate pellet. Unanesthetized fish were wrapped in a soft, wet cloth to facilitate holding. The other fish were anesthetized by placing them in a 15 C solution of 100 ppm M.S. 222 and leaving them until they were unable to remain upright but were still respiring freely (Stage I, Houston and Woods 1972). This generally required 2–3 min. The pellet was placed with the DDT side pointing into the fish's esophagus and pushed with a glass rod into the stomach. Preliminary trials indicated the food pellet was not regurgitated with this method. The fish were then placed in fiberglass tanks with aerated, filtered, running water at 15 ± 1 C, all fish of a given dose in one tank. Tests were conducted over a period from October, 1972 to February, 1973. Testing times for each group were systematically randomized throughout the study to offset the effect of time of day (Hatfield 1970) or season on conditioning. The times of DDT administration were staggered to correspond to the test time 48 or 72 h later. For each 1.0-tu test, two fish were treated to compensate for the expected deaths of half the fish, and one chosen at random and tested if both survived.

The conditioning apparatus (fully described in McNicholl 1974) was a T-shaped plexiglass box fitted with stainless steel grids through which a 2.1 m amp AC 60 cps shock could be delivered. The fish could move freely among the three compartments (Fig. 1). The fish was placed in compartment 3, given 10 min to become accustomed to the apparatus, then gently pushed into compartment 2. After 30 s the light (Fig. 1) (723 lx) was turned on for 10 s and the fish shocked if it remained in compartment 2 or went to compartment 1. If it went to compartment 3 the light was turned off. It was then gently pushed back into compartment 2, given a 30-s rest, and the sequence repeated. To eliminate fish with a strong

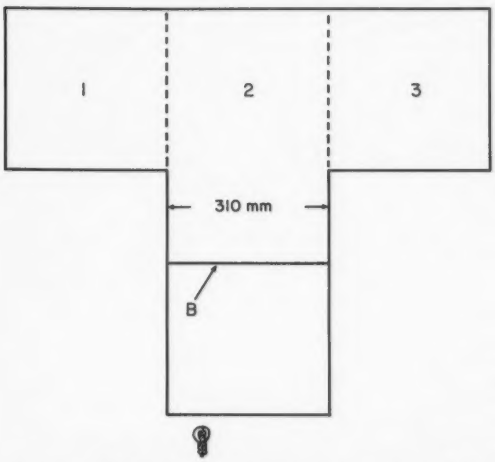

FIG. 1. The testing apparatus. 1, 2, 3 = areas of the box. B = barrier. The light is indicated by the symbol at the base of the T.

left or right preference, a fish that did not make the correct response in four to six inclusive of the first 10 trials was discarded, i.e. fish making less than four and more than six correct responses were discarded. Because of this elimination of fish with a strong preference, and the fact that each fish was tested only once, it was considered unnecessary to reverse the positions of compartments 1 and 3 between fish. The experimenter sat beside compartment 3 so all fish were exposed to the same external stimuli.

Each trial was recorded as either an error (a shock) or correct. Once a fish made 10 consecutive correct responses it was considered to have reached criterion. Two measures were taken of learning rate; trials to criterion was the total number of light presentations until the tenth consecutive correct response was observed, and errors to criterion was the number of shocks given in this same number of trials. Total testing time was 30–45 min per fish.

Fish that were disorientated or lost equilibrium prior to or during testing were discarded. This occurred only in the 1.0-tu group and was taken as an indication of near death.

Results

The 96-h LD50 from the lethality tests was 0.03 mg DDT/g body weight. Fish generally died 24–48 h after DDT administration. No delayed mortality occurred; thus, the 96 h-LD50 is likely the incipient LD50 (as in Sprague 1969). Use of this dose in the 1.0-tu group in the conditioning experiment resulted in an overall death rate of 45% (40 of 89 fish) which is not significantly different from 50% mortality ($Z = 0.79$).

TABLE 2. Mean number of trials to criterion and errors to criterion for pooled data at each dose.

Group[a]	N	Mean trials to criterion ± 1 SE	Mean errors to criterion ± 1 SE
control	32	31.2±1.3	9.0±0.4
0.1 tu	32	28.4±1.2	8.3±0.5
1.0 tu	32	23.7±0.8[b]	6.4±0.4[b]

[a]Where control = pooled data for all controls etc.
[b]Significantly different from controls, $P < 0.01$.

The number of trials and errors to criterion for each fish were recorded, and group means were determined (Table 1). An analysis of variance on the 12 groups showed that doses of DDT significantly affected the number of trials and errors to criterion ($P < 0.001$), but time of testing (48 or 72 h) after DDT administration or use of M.S. 222 did not. Therefore, data from a given dose were pooled (Table 2). A Duncan's Multiple Range test showed that DDT at the 1.0-tu level significantly decreased the number of trials and errors to criterion ($P < 0.01$), but at the 0.1-tu level, DDT had no significant effect. It was considered that a decrease in the number of trials or errors to criterion indicated an increase in learning rate. Although the 0.1-tu group was not significantly different from the controls, a dose response curve is indicated by their slightly improved response.

Discussion

In the present study DDT at the LD50 dose increased the rate of learning as measured by both number of trials and errors to criterion. Previous studies concerning effects of DDT on learning in fish have produced contradictory results. Anderson and Prins (1970) showed that brook trout (*Salvelinus fontinalis*) exposed to sublethal DDT levels either could not be conditioned to exhibit the propeller-tail reflex or required significantly more trials than the controls. Anderson and Peterson (1969) found that brook trout trained by electric shock to avoid the side of their preference in a two-chambered aquarium could not be conditioned if given DDT. However, Jackson et al. (1970) demonstrated that this was due mainly to the water level in the apparatus and DDT-treated fish could learn as well as the controls if the water level were lowered. Hatfield and Johansen (1972) using a similar apparatus found that DDT-treated salmon (*Salmo salar*) at the 96 h-LC50 level learned significantly better

than the controls, whereas those given 10% of the LC50 dose did not differ significantly from the controls. The latter treatment did show scattered improvement, however, and the average number of trials to criterion appears to be less than that of the controls (Hatfield and Johansen 1972, fig. 2C). Thus, the results of the present study agree with those of Hatfield and Johansen (1972), the major difference being the use of an oral dose of DDT in this study rather than suspending DDT in the water.

The results of the present study indicate that M.S. 222 does not influence learning at 100 ppm, a level commonly used for anesthetization (Wedemeyer 1970 used 80 ppm, Houston et al. (1971) used 100 ppm). This was true both in control and DDT-treated fish. Studies by Wedemeyer (1970) and Houston et al. (1971), for example, show diverse physiological effects of M.S. 222 but no work has been done to date on its effects on learning. Goddard et al. (1974) in fish more fully anesthetized than those in the present study found behavioral changes in M.S. 222-treated fish exposed to a temperature gradient. On the basis of these results they recommended that anyone using M.S. 222 prior to behavioral studies wait at least a week to avoid aftereffects of the anesthetic. The present study does not support this recommendation as M.S. 222-treated fish tested only 48 h after treatment showed no effect of the anesthetic. The difference in results may be due either to the difference in M.S. 222 dose or in the type of behavioral response measured.

More important is M.S. 222's lack of synergistic effects with DDT. Because of its widespread use as an anesthetic, both in research and in hatchery practice (Schoettger et al. fide Wedemeyer 1970), and the equally widespread appearance of DDT and other hydrocarbon residues (e.g. Reinert 1970 and Henderson et al. 1971), it is noteworthy that, at least for this parameter, there is no measurable interaction.

According to Macek and Korn (1970) fish in nature obtain most of their DDT from the food chain. However, Grzenda et al. (1970) found the water to be the most important source of DDT. Therefore, both sources are likely important to the fish. The effects on behavior in fish of waterborne DDT have been extensively studied; the use of an oral dose in the present paper has looked at a previously unexamined effect of DDT. The results of feeding fish a single, large dose of DDT given in the manner indicated in this paper would be analagous to a fish eating large numbers of insects that have been killed by spraying with DDT (Surber 1946; Ginsburg 1947; Hoffman and Linduska 1949; Hoffman and Surber 1949; Janzen 1960). Due to the persistence of the DDT and its metabolites in the body tissues of the fish (Gakstatter and Weiss 1967; Macek et al. 1970), the effects would likely persist for some time, as much as 160 days later (the half-life of DDT, Macek et al. 1970). This is seen in the present study where similar effects of DDT were seen 48 and 72 h after treatment. Long-term effects could not be evaluated in the present study due to the lack of adequate holding facilities.

In conclusion, a single oral dose of DDT was shown to increase the rate of learning only at a level that killed half the fish. Thus its possible benefits to the individual are outweighed by the obvious disadvantages to the population.

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Aspects of the Winter Ecology of Juvenile Coho Salmon (*Oncorhynchus kisutch*) and Steelhead Trout (*Salmo gairdneri*)

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The major physical characteristics of overwintering areas for juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*) are described for a small, unlogged, west coast Vancouver Island stream. During the winter months age 1+ coho and steelhead were found at a range of depths mainly greater than 45 cm and in deeper water than age 0 of either species. About 45% of age 0 steelhead observed were in water <15 cm deep. The depth occupied by coho and age 1+ steelhead was negatively correlated with water temperature below 8.5 C. Coho were associated less closely with the bottom than were steelhead. At 7 C or less most fish were associated with water velocities of <15 cm/s. Velocities in which steelhead occurred were positively correlated with rising temperature above 4 C. As water temperature decreased from 9 to 2 C, coho and steelhead moved closer to cover. Cover used by coho and age 1+ steelhead most frequently was logs and upturned tree roots, although debris accumulations and overhanging banks were also used. Both age-groups of coho used overhanging brush but steelhead did not. Over 50% of age 0 steelhead were associated with rocks 10–25 cm in diameter. Sidepools and quiet back channels that contained water only in the winter and that had combinations of the above cover types were populated by coho during the winter. A series of unused beaver ponds, dry in the summer, was an important overwintering area for coho with a survival rate about twice as high as the 35% estimated for the entire stream system.

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Nous décrivons les principaux caractères physiques des aires d'hivernage de jeunes saumons coho (*Oncorhynchus kisutch*) et truites steelhead (*Salmo gairdneri*) dans un petit cours d'eau, libre d'exploitation forestière, de la côte occidentale de l'île Vancouver. Pendant les mois d'hiver, les saumons coho et truites steelhead d'âge 1+ se trouvent surtout à des profondeurs dépassant 45 cm et en eau plus profonde que les poissons d'âge 0 de l'une ou l'autre espèce. Environ 45% des truites steelhead d'âge 0 observées se trouvent à des profondeurs de <15 cm. La profondeur occupée par les saumons coho et par les truites steelhead d'âge 1+ est en relation inverse de la température de l'eau au-dessous de 8.5 C. Les saumons coho sont moins étroitement associés au fond que ne le sont les truites steelhead. A 7 C ou moins, la plupart des poissons se trouvent dans des courants de <15 cm/s. Les vitesses où se rencontrent les truites steelhead sont en relation directe d'une température ascendante au-delà de 4 C. A mesure que la température diminue de 9 à 2 C, les saumons et les truites se rapprochent d'un abri. Les abris le plus souvent utilisés par les saumons coho et les truites steelhead d'âge 1+ sont des billots ou des racines d'arbre renversé, alors que des tas de débris et des berges en surplomb sont également utilisés. Les deux groupes d'âge de saumons coho utilisent la brousse qui surplombe, mais il n'en est pas ainsi de la truite steelhead. Plus de 50% des truites steelhead d'âge 0 sont associées à des roches de 10–25 cm de diamètre. Les fosses latérales et les chenaux de retour tranquilles contenant de l'eau seulement en hiver et combinant les mêmes types d'abri que ci-haut sont peuplés par le

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saumon coho en hiver. Une série d'étangs de castors abandonnés, à sec en été, sont une aire d'hivernage importante pour le saumon coho, avec un taux de survie d'environ le double du 35% estimé pour le réseau entier du cours d'eau.

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In coastal British Columbia juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead (*Salmo gairdneri*) reside in streams for 1 or more years before migrating to sea. Much of this time in the stream is spent during the winter when physical conditions are severe. At this time salmonid nursery streams are characterized by low water temperatures accompanied by snow, ice, or severe freshets. Injury or death can result from inadequate nutrition (Hunt 1969; Reimers 1963), severe streamflow fluctuations causing scouring and displacement (Elwood and Waters 1969), collapsing snow (Needham and Jones 1959), or predation (Elson 1962). Important questions are, how do juvenile salmonids adapt to these hostile winter conditions, and how might man's activities, particularly timber harvesting, affect these overwintering fish?

In some streams the major factor limiting coho production may be the amount of adequate overwintering habitat rather than living space in low summer flow (Mason 1974). Some preliminary underwater observations by us in several small coastal streams during 1972 supported conclusions by Hartman (1965) and Everest (1969) that young salmonids occupy different habitats in the winter than in the summer, and that log jams and rubble provide important winter cover.

The objectives of this study were:

1) To describe quantitatively some of the major physical characteristics of overwintering areas in a coho and steelhead nursery stream.

2) To evaluate the role of a small tributary of this nursery stream in providing winter refuge for young coho.

Study Area

Carnation Creek, a small coastal British Columbia coho and trout nursery stream, and one of its tributaries (750 Creek), was studied (Fig. 1). The Carnation Creek watershed is approximately 10 km² and drains into Barkley Sound on the west coast of Vancouver Island. It is the site of a long-term study examining the effects of timber harvesting on salmonid production (Anon. 1974). The watershed consists of an unlogged, overmature western hemlock (*Tsuga heterophylla*), amabalis fir (*Abies amabalis*), and western red cedar (*Thuja plicata*) forest with Sitka spruce (*Picea sitchensis*), red alder (*Alnus rubra*), salmonberry (*Rubus spectablis*), and stink currant (*Ribes bracteosum*) common along the streams.

The watershed is in a region of high annual precipitation (275–450 cm), with most of it occurring as rainfall between October and April. Although streamflow ranged from 0.03 m³/s (1 cfs) during late summer to 29 m³/s (1000 cfs) during peak winter freshets, violent fluctuations within short time periods are characteristic of streamflow during autumn, winter, and spring.

Water temperatures in Carnation Creek reach a maximum of 13.5 C in the late summer and drop to near 1 C for short periods during the winter. Some surface ice forms in back channels during the coldest periods, and water temperatures remain less than 6 C from December through April.

The section of Carnation Creek that was examined in this study drops approximately 60 m in the lowest 2.5 km. The substrate, ranging from sand to 30 cm rubble, consists predominantly of rocks 2–15 cm in diameter. Extensive gravel bars suggest that considerable substrate movement occurs during freshets. Wind-felled trees with upturned roots, overhanging banks, and small accumulations of debris are common throughout the study area.

A tributary, 750 Creek, enters Carnation Creek 750 m above the head of tide and consists of a series of interconnected, mud-bottomed pools formed behind old, deserted beaver dams. It drains an area of approximately 14 ha. Winter flows are generally less than 0.03 m³/s (1 cfs), but may range as high as 0.2 m³/s (7 cfs) during freshets. The lowest 100 m of the creek is utilized by young salmonids at higher flows but is dry during the late summer. Adjacent vegetation consists primarily of western hemlock, and a dense understory of salmonberry, and tall blue huckleberry (*Vaccinium ovalifolium*).

The lowest 3.5 km of Carnation Creek is accessible to anadromous fish, and is utilized by coho and chum (*O. keta*) salmon, steelhead and cutthroat trout (*S. clarki*) as well as resident populations of two species of sculpins (*Cottus aleuticus* and *C. asper*). The upper section of the stream supports a population of resident cutthroat trout. Coho reside in the stream 1 or 2 yr and steelhead 2–4 yr (mainly 3) before migrating to the ocean (Narver and Andersen 1974). In this paper age 0 refers to fish of the year (fry) and age I+ are fish in their second year of life (yearlings) or older.

Some winter snorkle observations were also made in the Sarita River and Ritherdon Creek both adjacent to Carnation Creek (Fig. 1) and in Paton Creek, a tributary of the Seymour River near Vancouver, B.C.

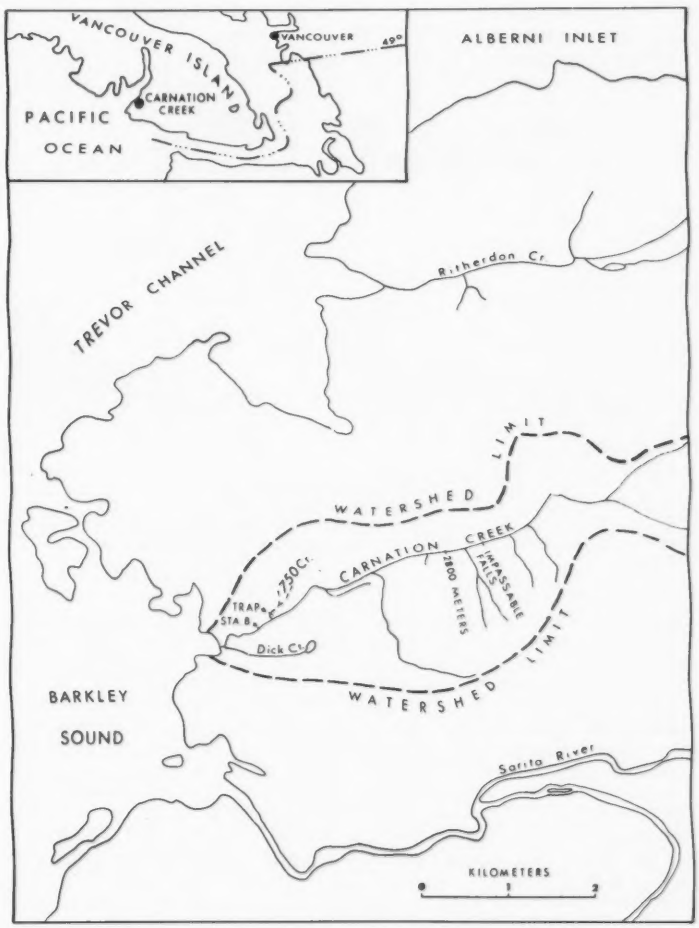

FIG. 1. Location of the Carnation Creek watershed and study sections (Scale: 1:50,000).

Methods

Coho and steelhead microhabitat information was collected by making observations while snorkeling with wet suit and face mask in the lowest 2800 m of Carnation Creek from September 1972 to April 1973. An observer entered the water and carefully worked upstream until an undisturbed fish or aggregations of fish were observed. Often the observer was on or near the bottom when observations of fish were made. In deeper water the observer was floating but observations were made from 3 to 6 m downstream. An underwater light was used for searching in log jams and other poorly lighted areas. A metal rod was used to overturn rocks and probe for fish within the substrate. Fish were visually separated by species and age-group (0 and I+). Observations were made between 1000 and 1600 and were restricted by fast currents and poor visibility to streamflows less than 3 m^3/s (105 cfs).

After an individual fish was observed for 1–5 min, its most frequent location or focal point (Wickham 1967) was marked with a lead weight. Coho were often found in close aggregations and a count of the number of fish in the aggregation was obtained. Water depth, velocity, size of substrate material, distance to suitable cover, and type of cover was recorded for each observation. A metal rod marked in

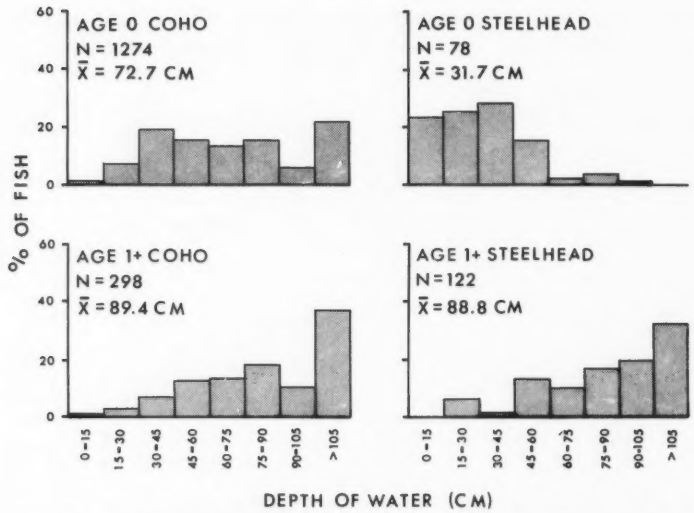

FIG. 2. Depth of water selected by juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*) at water temperatures of 7 C or less.

centimeter intervals was used for measuring water depth, cover distance, and substrate size. Water velocities, both focal point and maximum area (within 0.3 m of the point of observation for age 0 fish and 0.6 m for age I+ fish), were measured to the nearest centimeter per second with Midget Bentzel current speed tubes (Everest 1967). These instruments were suitable for measuring currents between 15 and 120 cm/s. Stream discharge (weir measurements) and water temperatures (recording thermograph) were obtained at the beginning and end of each observation period from a permanent hydrological installation maintained at Station B (Fig. 1). Upturned tree roots, logs, overhanging banks with and without roots, overhanging brush, debris (i.e. small, temporary accumulations of branches and snags), rubble, and turbulent surface water constituted potential areas of cover. Fish more than 2 m from cover were assigned to a "no cover" category.

Snorkeling has been used by other workers while examining young salmonid distribution in relation to their habitat (Hartman 1965; Edmundson et al. 1968; Everest 1969; Griffith 1972). A major difference in making summer and winter snorkel observations was the greater tendency for fish in the winter to flee an approaching swimmer. This pronounced fright reaction necessitated very slow, careful movements when approaching fish. Juvenile fish during the summer are usually more aggressive, less easily frightened, and therefore easier to observe. In the winter, hiding fish tend to seek the darkest areas available, twisting behind roots and taking on the color of their surroundings, making them difficult to find. Observations are also limited by high flows and associated turbidities. Finally, when water temperatures are less than 4 C, an observer in a wet suit experiences severe cold, sometimes after less than ½ h in the stream.

A trapping system near the outlet of 750 Creek (Fig. 1) measured upstream and downstream fish movements. A wire mesh (6 mm) fence diverted upstream and downstream migrants into small fyke traps. Rocks were placed in the traps to provide escape cover for the smaller fish. The traps were checked daily. All fish were anesthetized, fork length was recorded, and after recovery fish were released in the direction indicated by their capture.

Results

Coho were more common than steelhead in the study section; observations were made on 1311 age 0 and 351 age I+ coho, and 122 age 0 and 167 age I+ steelhead.

Water Depth and Velocity

There were major differences in the mean depths of water (surface to bottom) occupied by juvenile coho and steelhead during the winter (Fig. 2). Age 0 coho were found in a wide range of depths greater than 15 cm. Age I+ coho and steelhead also occupied a variety of depths, but favored significantly deeper water than age 0 coho (t-test, $P < .01$). Age 0 steelhead were strongly associated with shallow water, often in less than 15 cm. A

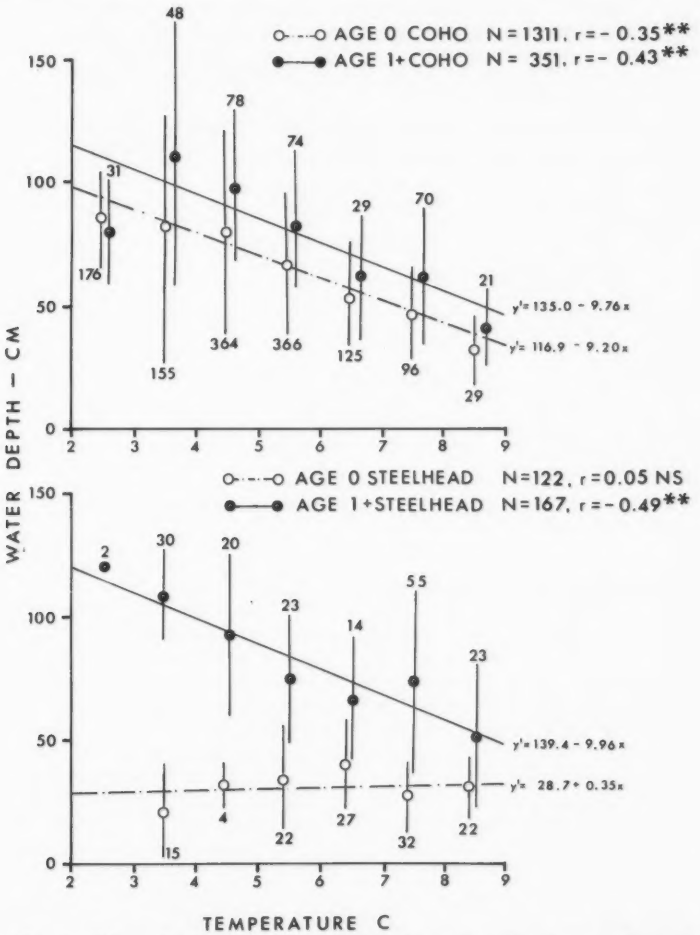

FIG. 3. Mean depth of water in which coho and steelhead were observed related to stream temperatures at time of observation. Vertical lines represent standard deviations and associated numbers are sample sizes. Regression lines were derived from N observations.

number of fry were found under rocks in water less than 5 cm deep.

Significant negative correlations ($P < .01$) were found between water temperatures below 8.5 C and water depth occupied by coho and age I+ steelhead (Fig. 3), indicating that as water temperatures drop in the late autumn and early winter these fish tend to move into deeper water. No significant correlation existed for the age 0 steelhead.

Coho usually associated less closely with the stream bottom than steelhead; their average distance from the stream bottom was 25 and 27 cm for age 0 and age I+ coho, respectively. The average distance above the stream bottom was 5 cm for age 0 and 12 cm for age I+ steelhead.

At low water temperatures 78–87% of all fish were found in focal point velocities of less than 15 cm/s (Table 1). Similarly 58–78% of the fish were in maximum area velocities less than 15 cm/s (Table 1).

For age 0 and I+ steelhead, focal point and maximum velocities in surrounding areas increased significantly with rising temperature above 4 C

TABLE 1. Breakdown into velocity categories of focal point and maximum area water velocities selected by juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*) at water temperatures of 7 C or less expressed as a percentage of all observations.

	Coho		Steelhead	
Age	0	I+	0	I+
Sample size	1272	298	78	122
	Focal point velocities			
Velocity (cm/s)				
0–15	86.8	84.7	87.1	78.0
15–30	13.1	14.7	11.6	22.0
30–45	0.1	0.6	1.3	0
>45	0	0	0	0
	Maximum area velocities[a]			
0–15	77.8	66.4	57.6	62.7
15–30	18.3	21.8	26.9	16.9
30–45	3.8	8.0	9.0	11.8
>45	0.1	3.8	6.5	8.5

[a]Refers to the maximum water velocity within 0.3 m for age 0 fish and within 0.6 m for age I+ fish.

(Fig. 4). Only measurements taken during moderate streamflows (0.3–0.7 m^3/s) are included because of apparent variability in fish activity relating to very low or high flows. The scatter around these means is very broad. These mean velocities do not represent the true mean velocities; because the current meters were of low sensitivity, velocities less than 15 cm/s were recorded as nil. Thus results should be used to indicate general relationships rather than actual values. No significant relationship between water velocity occupied by coho and water temperature was indicated other than at water temperatures less than 4 C when the mean water velocities selected by coho were between 0 and 15 cm/s.

WINTER COVER SELECTION

As water temperature decreased from 9 to 2 C, coho and steelhead moved closer to objects providing cover (Fig. 5). The observations were broadly scattered around the means. When water temperatures became low (usually accompanied by low water), feeding activity was reduced, and nearly all fish were found either within or very near cover (Fig. 6). These fish were heavily pigmented and strongly photonegative.

Upturned tree roots and logs were the most common type of cover used by both age-groups of coho and by age I+ steelhead (Fig. 7). Deep pools were often formed around these roots and logs, and the combination of deep, slow water and good cover made these sections of the stream important wintering areas, particularly for the older fish. Overhanging banks and debris such as branches and small snags were also used as cover. However, only those overhanging banks that had an associated complex of dense roots were utilized. Flooded brush such as salmonberry and stink currant provided some cover for coho, especially in backwater pools and tributary areas.

Sidepools combining a number of cover types described in Fig. 7 provided coho refuge from severe winter freshets. For example, in early January 1973 we electrofished a sidepool (approximately 10 m^2 with a maximum depth of 25 cm) located 50 m from the main stream and captured 54 coho which were removed. When electrofished again in early March, another 20 coho were found. During the summer this pool is dry.

Rubble was the principal source of cover for age 0 steelhead, and half of the fry observed at water temperatures of 7 C or less were either very close to or under rubble (Fig. 7). Of steelhead fry found in the rocky substrate, more than half were found under rocks less than 15 cm, and rocks as small as 10 cm provided cover as long as there were suitable interstices (Fig. 8). These overwintering sites were usually in shallow areas of low velocity near the stream margin (Fig. 8). In several instances two fry were found under the same rock.

Only four age I+ steelhead were found hiding

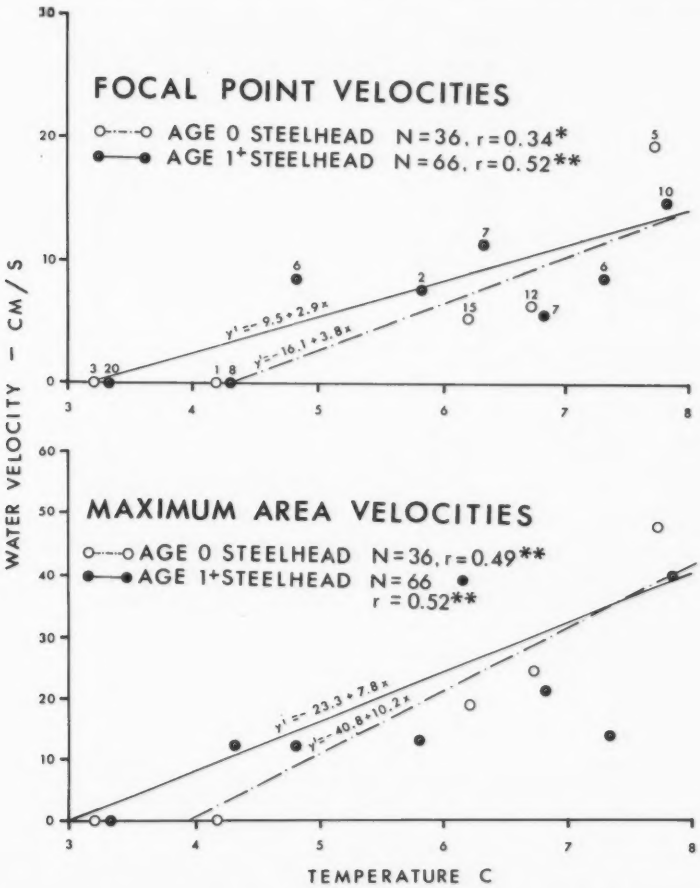
FOCAL POINT VELOCITIES
AGE 0 STEELHEAD N=36, r=0.34*
AGE 1+STEELHEAD N=66, r=0.52**
y' = - 9.5 + 2.9x
y' = - 16.1 + 3.8x
MAXIMUM AREA VELOCITIES
AGE 0 STEELHEAD N=36, r=0.49**
AGE 1+STEELHEAD N=66
r = 0.52**
y' = - 23.3 + 7.8x
y' = - 40.8 + 10.2x
WATER VELOCITY - CM/S
TEMPERATURE C

FIG. 4. Mean focal point and maximum area water velocities selected by age 0 and age I+ steelhead in relation to water temperature. Streamflows were between 0.3 and 0.7 m^3/s for all measurements. Sample size is indicated by the associated numbers. Regression lines were derived from N observations.

under rubble, and none were more than 70–80 mm in length. Age I+ steelhead were commonly found under boulders in the Sarita River. Older age-groups of cutthroat trout were found under boulders as well as log jams, upturned roots, and debris accumulations in upper Ritherdon Creek. Very few coho were found under rubble in Carnation Creek, although coho fry did utilize rubble for winter cover in Paton Creek.

FEEDING AND HIDING BEHAVIOR

When water temperatures were above 7 C, most fish in Carnation Creek were active and feeding. In water between 4 and 7 C coho and steelhead varied between being active and inactive depending on stream conditions. For example, at water temperatures of 7 C and streamflows of 0.03 m^3/s (1 cfs), virtually no steelhead fry could be observed above the substrate. With increased streamflow, steelhead fry were observed maintaining positions in moderate currents and actively feeding, even though the water temperature was 6 C.

During January and February, the period of coldest water, few coho and steelhead were ob-

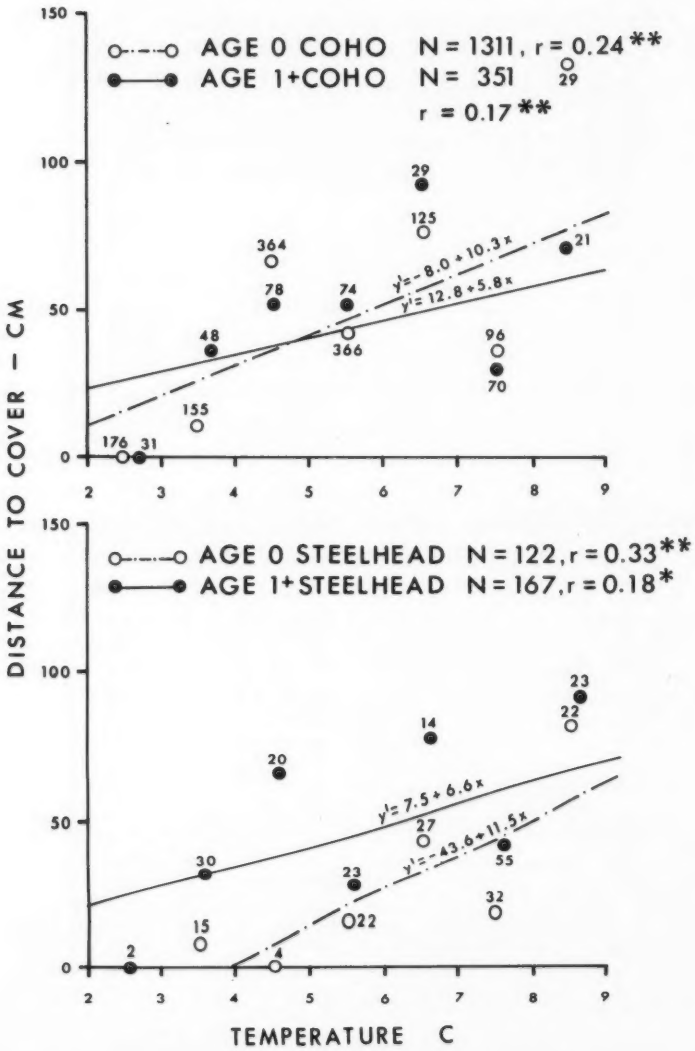
AGE 0 COHO N = 1311, r = 0.24**
AGE 1+COHO N = 351
r = 0.17**
y' = -8.0 + 10.3x
y' = 12.8 + 5.8x
AGE 0 STEELHEAD N = 122, r = 0.33**
AGE 1+STEELHEAD N = 167, r = 0.18*
y' = 7.5 + 6.6x
y' = -43.6 + 11.5x
DISTANCE TO COVER - CM
TEMPERATURE C

FIG. 5. Mean distance to cover of juvenile coho and steelhead in relation to water temperature. Sample size is indicated by the associated numbers. Regression lines were derived from N observations.

served in open areas. Only after intensive searching with a light a few fish could be located far under logs and undercut roots. Coho were more active at low water temperatures than steelhead, and some were observed feeding at water temperatures as low as 2.5 C.

No juvenile steelhead were ever observed feeding at low water temperatures in the Sarita River where the fry entered the rocky substrate in early October at water temperatures between 7 and 8 C and remained there into the following spring. Sarita River steelhead in the older age-groups appeared to enter the substrate later than the fry.

TRIBUTARY MOVEMENTS

The 750 Creek system was used primarily by juvenile coho although several dozen sculpins and

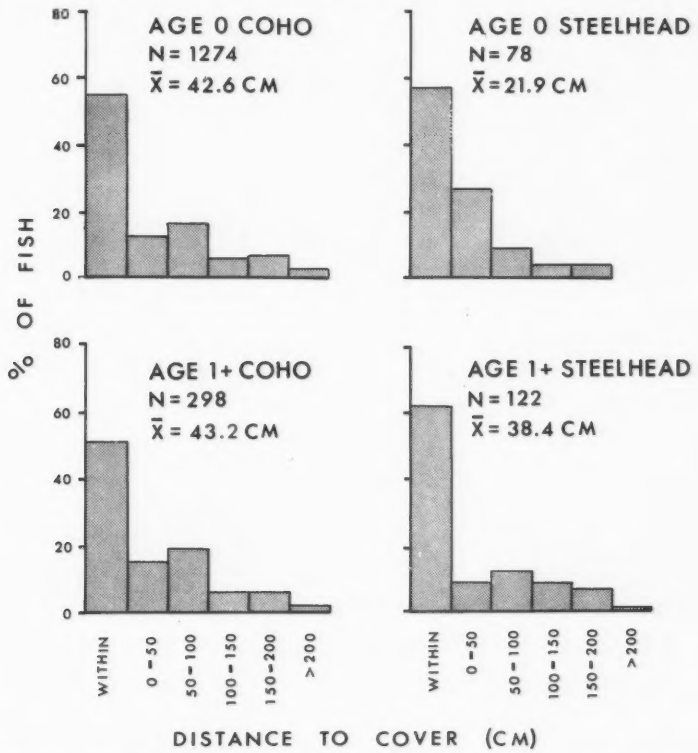

FIG. 6. Distance to nearest cover from juvenile coho and steelhead at water temperatures of 7 C or less.

three trout moved into it during the fall and winter. Monthly totals of the movements of coho through the traps are presented in Fig. 9. The traps were inoperative one day in December because of high water. Except for a few isolated pools, 750 Creek was dry throughout most of September and all of October. A small number of coho moved into the creek during a freshet in late September. Upstream movements occurred predominantly in November and December, whereas steady downstream movements occurred from February through May with a peak of over 60 coho in 1 day during a freshet in late May. Most movements were associated with freshets and water temperatures greater than 6 C. During the entire period 358 coho moved into 750 Creek and 266 left — a gross estimate of survival of 74%. A more meaningful estimate is obtained by considering only the net monthly movements which total 238 up and 145 down — 61%. During September to December mean length was 54.1 mm for upstream fish and 61.5 mm for downstream. The length of migrants from January to May was 62.5 mm for upstream fish and 64.5 mm for downstream.

Discussion

Beginning in early October, juvenile coho and steelhead in Carnation Creek undergo a behavioral change in response to approaching winter stream conditions. With decreasing water temperatures, these fish tend to be less active and spend much of their time hiding. This increased hiding with lower water temperatures appears to be characteristic of juvenile salmonids and has been discussed in some detail by Chapman and Bjornn (1969). Water depth and velocity and the availability of cover are important in determining the suitability of stream areas for these hiding fish.

Influence of Temperature

Observations throughout autumn and winter in Carnation Creek suggest that shorter day length

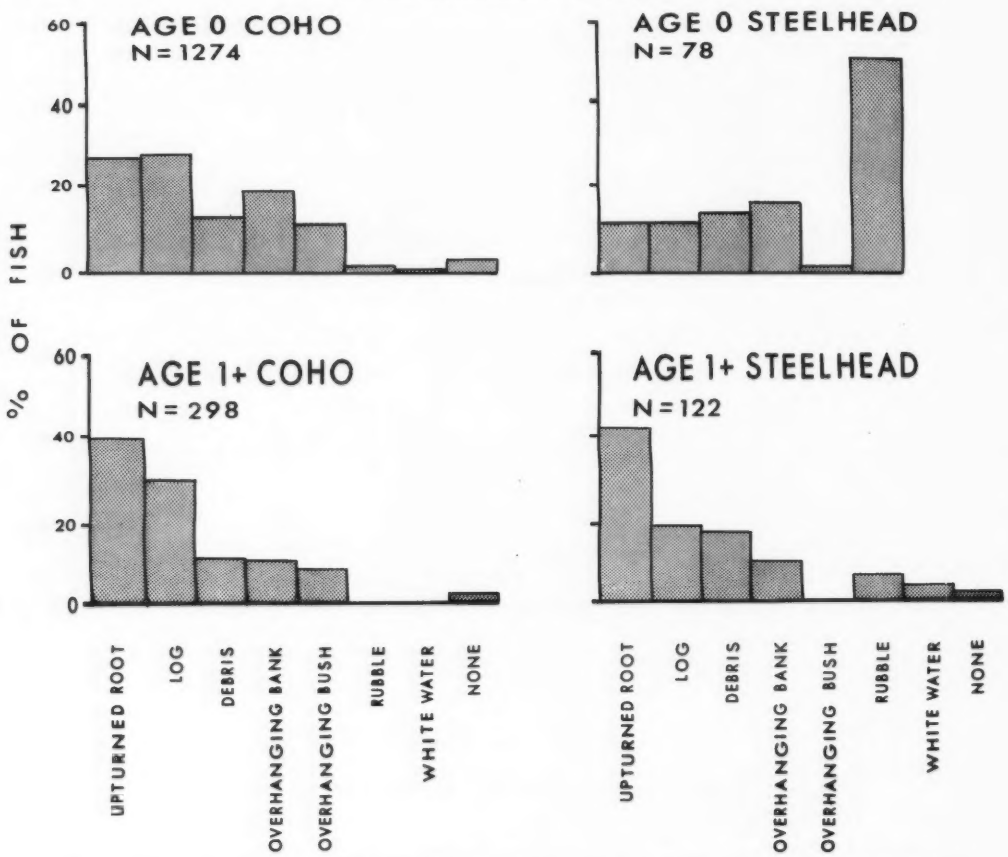

FIG. 7. Cover types selected by juvenile coho and steelhead at water temperatures of 7 C or less.

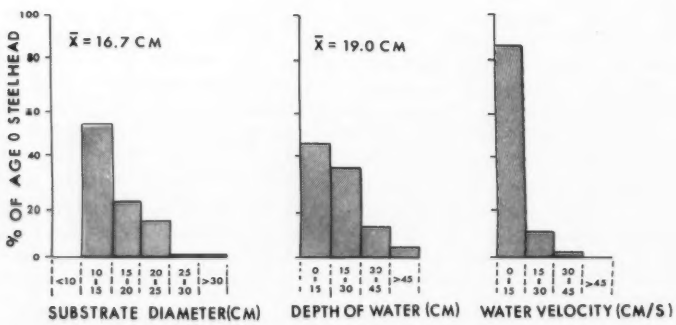

FIG. 8. Physical characteristics of substrate wintering areas selected by 43 age 0 steelhead.

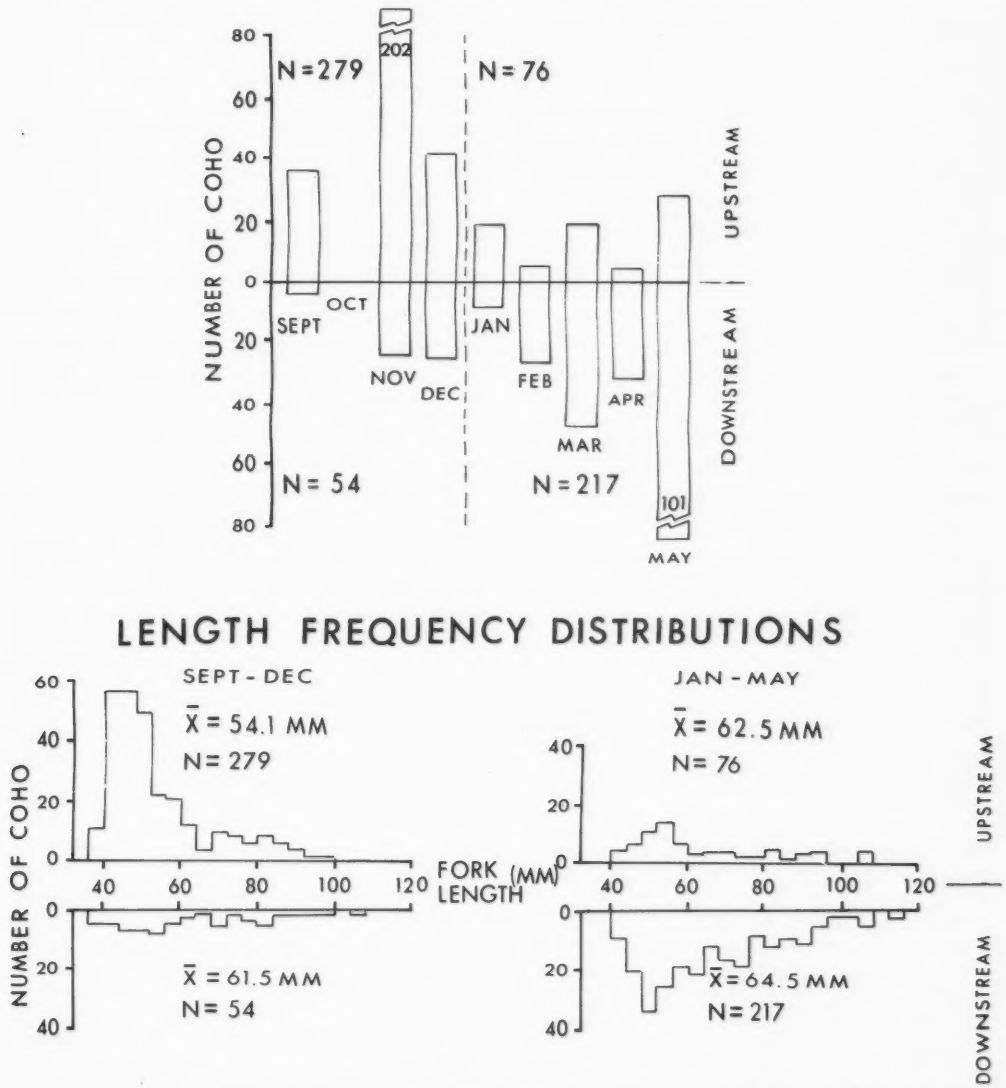

FIG. 9. Monthly upstream and downstream movements and length frequency distributions of juvenile coho in 750 Creek. Totals from spring do not include recently emerged fry.

did not have an important influence on the hiding behavior of juvenile coho and steelhead. For example, many of the steelhead fry which were hiding in the substrate in mid-October were actively feeding in November and early December. Hartman (1965) was able to induce steelhead and coho to hide in a stream aquarium by lowering the water temperature even though he maintained day length at a constant 12 h. Similarly, by maintaining a constant 13-h day length, Chapman and Bjornn (1969) showed that the number of yearling steelhead hiding in rubble cover was directly related to water temperature.

Although temperature was a major factor in-

ducing a hiding response in fish in Carnation Creek, stream discharge also appeared to influence fish activity, especially at water temperature between 4 and 8 C. Increased activity may reflect a greater availability of food organisms than at lower temperatures and/or an increase in protection from predation provided by faster and more turbid water. More definitive investigation of the physiological and enviromental parameters controlling wintering behavior of juvenile salmonids is necessary to explain some of our observations.

Water Depth and Velocity

As water temperature dropped during the fall and early winter, coho and older steelhead in Carnation Creek moved into deeper water (Fig. 3). In stream aquarium experiments, underyearling coho and steelhead showed a strong preference for the deepest sections at lowest water temperatures (Hartman 1965). However, Everest (1969) suggests that water depth and overhead velocity were not important in determining steelhead overwintering areas. Most steelhead fry in Carnation Creek chose shallow areas near the stream margin for overwintering in the rubble substrate. For fish that do not enter the substrate in the winter, such as most of the coho and older steelhead in Carnation Creek, deep pools offer the advantages of cover and reduced water velocities. Steelhead fry overwintering within the rubble substrate may obtain these advantages from overhead rocks.

In the small rubble substrate typical of Carnation Creek, steelhead fry were not found as deep as has been reported in other river systems (Edmundson et al. 1968; Everest 1969). Some of the shallow areas used by the steelhead fry in Carnation Creek were dry during low winter flows. Fry hiding in these areas must shift location with changing stream discharge. Everest (1969) suggests that once fish move into the rocky substrate for the winter, no subsequent movements occur. In colder streams with less fluctuating winter flows than Carnation Creek, such as Everest's Idaho streams, this may be the case.

Steelhead of both age-groups occupied areas of lowest water velocity with decreasing temperature (Fig. 4). Such a relationship was not demonstrated for coho, probably for two reasons. First, most coho tend to occupy pool areas of lower velocity the year around. Therefore, the change in selected water velocities with decreasing water temperatures was not as obvious as for steelhead. Secondly, the current meters were not sensitive enough to detect differences between the "low velocity" feeding areas and the "no velocity" hiding areas.

Winter Cover

At low water temperatures, suitable cover areas appeared to be critical and few fish were found more than 1 m from potential cover (Fig. 5 and 6). Similar observations were made by Hartman (1965). Our observations also agree with Hartman's that steelhead fry and coho fry seek different cover types in the winter, with coho associated with logs, roots and bank cover areas and steelhead associated with rubble areas (Fig. 7). Older steelhead often selected the same types of cover as coho in Carnation Creek, but they were usually found closer to the bottom than coho, in deeper pools than the coho fry, and almost never associated with sidepools or bay areas as many of the coho were.

Steelhead fry utilized different winter cover than age I+ steelhead in Carnation Creek. Rubble in the 10–25 cm range was utilized by steelhead fry, whereas older steelhead hid in upturned roots and under logs and debris (Fig. 7). Everest (1969) indicated juvenile steelhead of several age-groups were found only under rubble larger than 40 cm in diameter, and Hartman (1965) found that most steelhead in the upper Chilliwack River were under 20–40 cm rocks. However, substrate of Carnation Creek was much smaller than in the above rivers. We commonly found older age-groups of steelhead and cutthroat in the much larger rocks of Sarita River and Ritherdon Creek.

Most juvenile steelhead spend two or three winters in Carnation Creek before smolting. Based on 3-yr data, survival from the late summer fry to midsummer age I is about 6% (range 5–13%). These low survival rates point to the winter–spring period as crucial to steelhead during their stream residence. This probably reflects the low availability of suitable winter cover in Carnation Creek. Steelhead fry used small rubble, often less than 15 cm in diameter, presumably because nothing larger was available. Such small substrate probably provides a hazardous refuge during winter freshets. C. Lyons (B.C. Fish and Wildlife Branch, personal communication) has found injured steelhead fry in late winter and suggests that injuries may have resulted from shifting substrate. Age I+ steelhead overwinter in large rubble and boulder areas in other streams, but in Carnation Creek where the substrate is mainly small rubble they used alternative (less suitable?) types of winter cover.

During the winter *Cottus aleuticus* were com-

monly found in pools under a similar rubble substrate as steelhead fry but often in deeper water. The two species were never found under the same rock, but in areas of limited rubble cover, competition during the winter might occur.

Tributary Movements

The overwinter survival of juvenile coho that moved into 750 Creek during the 1972–73 winter was considerably higher (61–74%) than the 35% estimated for coho in the whole Carnation Creek system. Presumably thick overhanging vegetation, fallen trees, and broad, slow pools provided protection from both predation and displacement during the winter. The survival of fish moving into small tributaries in the autumn and early winter is probably dependent on subsequent weather conditions. For example, dead coho fry were found in dried pools in 750 Creek during an extended period without rain following a September freshet. Coastal British Columbia winters are characterized by frequent major freshets, with presumed low coho survival in main streams. As indicated by the 750 Creek study, flooded side pools and small tributaries are probably the areas of highest coho overwinter survival. In March 1972, following a period of severe freshets, underwater observations indicated there were extremely low numbers of coho in the main Carnation Creek channel, especially in the lowest 1000 m. Sidepools and tributaries contained much higher densities of young fish.

Movements of juvenile coho in and out of 750 Creek is not unique. A similar autumn movement of juvenile coho into a tributary stream has been described by Skeesick (1970). In addition he mentions an October movement of 450 juvenile steelhead (60–200 mm in length) into a small tributary of the Elk River in Oregon. Large autumn movements of steelhead and chinook out of tributary streams with poor winter cover into larger streams with good boulder cover have been described by Chapman and Bjornn (1969) and Bjornn (1971).

Winter Hiding and its Adaptive Value

The hiding behavior demonstrated by juvenile salmonids in cold water has obvious adaptive value. A fish spending the winter in near freezing water temperature has a lowered metabolism, reduced food requirements, and less energy available for activity as discussed by Hartman (1968) and Reimers (1957). The hiding response is probably a means of avoiding predation during a period of low, clear water and reduced swimming ability. Another adaptive value of the hiding response is that it probably reduces downstream displacement during freshets.

Environmental Change

Juvenile coho and steelhead showed strong dependence on certain types of habitat during the winter. It appears that sidepools and back channels with logs, debris, and overhanging brush are important for coho. Age I+ steelhead and coho used logs, root masses, cutbanks, and debris for winter cover in the main stream. Age 0 steelhead used rocks at the stream margin. Most of this cover is associated with the streambank and is quite stable. We infer that streamside logging or road building, overzealous stream clearing, or channelizing could severely alter the salmonid winter habitat as defined in this paper and would probably result in reduced overwinter survival.

Acknowledgments

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Preferences of Juvenile Coho Salmon (*Oncorhynchus kisutch*) and Cutthroat Trout (*Salmo clarki*) Relative to Simulated Alteration of Winter Habitat

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BUSTARD, D. R., AND D. W. NARVER. 1975. Preferences of juvenile coho salmon (*Oncorhynchus kisutch*) and cutthroat trout (*Salmo clarki*) relative to simulated alteration of winter habitat. J. Fish. Res. Board Can. 32: 681–687.

Winter habitat preferences of juvenile coho salmon (*Oncorhynchus kisutch*) and cutthroat trout (*Salmo clarki*) were tested by simulating conditions before and after stream disturbance such as might result from logging: (1) sidepools with or without an overhanging bank and roots, and (2) sidepools with clean or silted rubble substrate. Both coho and cutthroat demonstrated a strong preference for sidepools offering overhanging bank cover as opposed to those without bank cover. Similarly they preferred sidepools with clean rubble substrate as opposed to silted rubble. In both the bank and rubble tests, when given the option of either remaining in the sidepools or of moving into the stream, a greater percentage of the total number of coho and cutthroat originally in the sidepools remained in the pools with cover as opposed to those without cover. Coho utilized bank cover more readily than rubble cover whereas cutthroat used both bank and rubble cover.

BUSTARD, D. R., AND D. W. NARVER. 1975. Preferences of juvenile coho salmon (*Oncorhynchus kisutch*) and cutthroat trout (*Salmo clarki*) relative to simulated alteration of winter habitat. J. Fish. Res. Board Can. 32: 681–687.

Nous avons éprouvé les préférences d'habitat d'hiver de jeunes saumons coho (*Oncorhynchus kisutch*) et truites fardées (*Salmo clarki*) en imitant les conditions qui prévalent avant et après qu'un cours d'eau ne soit dérangé, comme par exemple à la suite d'une exploitation forestière: (1) fosses latérales avec ou sans berge et racines en surplomb, et (2) fosses latérales à substrat de moellons propres ou envasés. Le saumon coho et la truite fardée démontrent tous deux une forte préférence pour les fosses surplombées d'une berge formant abri, par opposition aux fosses dépourvues de berge surplombante. Ils préfèrent également les fosses latérales à fond de moellons propres, par opposition aux moellons envasés. Dans les essais impliquant les berges comme dans ceux des moellons, quand les poissons ont le choix soit de demeurer dans les fosses latérales, soit de se déplacer vers le cours d'eau, un plus fort pourcentage du nombre total de saumons coho et de truites fardées déjà présents dans les fosses y demeurent lorsqu'il y a abri que lorsque l'abri manque. Les saumons coho utilisent plus volontiers l'abri des berges que celui des moellons, alors que la truite fardée utilise les deux.

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IN British Columbia, as elsewhere in North America, fisheries resources and logging practices are often in conflict. These conflicts frequently revolve around the value of streambanks to certain species of salmon and trout and how logging should be conducted to protect these values. The dependence of salmonids on a healthy, near-stream terrestrial environment during the growing season has been recognized (Mundie 1969; Chapman 1966). Juvenile salmonids occupy different habitat areas in the winter than in the summer (Hartman 1965; Everest 1969). Our observations indicate that the streambank environment is particularly important to overwintering juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*) (Bustard and Narver 1975). Underwater observations in several small

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coastal streams indicated that extensive use was made of marginal bays and sidepools by juvenile coho and of rubble substrate by juvenile steelhead and cutthroat trout (*Salmo clarki*). Because many of these areas are dry during the summer, they are easily overlooked and are highly susceptible to damage from timber harvesting.

The objective of this study was to test winter preferences of coho and trout for sidepool areas simulating conditions before and after stream disturbance. The simulated habitat types were: (1) sidepools with or without an overhanging bank and roots, and (2) sidepools with clean or silted rubble substrate. Elimination of streambank cover and accumulation of sediment within the stream substrate are two potential problems arising from logging operations.

The experiments were conducted on Dick Creek, a small stream tributary to the estuary of Carnation Creek, on the west coast of Vancouver Island (Bustard and Narver 1975). The Dick Creek watershed is approximately 1.2 km² and contains a 1-ha lake in its headwaters. Discharge ranges from 2 m³/s (70 cfs) during winter freshets to a trickle in late summer. During the study period from October 1972 to March 1973, maximum and minimum water temperatures ranged from 9 to near 0 C. The lowest 500 m of the creek is accessible to fish. The substrate in this section ranges from gravel to rubble 10–30 cm in diameter.

Methods

Four experimental sidepools were constructed adjacent to a long riffle section located 300 m up Dick Creek. The walls and bottom of each sidepool were constructed from plywood 1.9 cm thick and placed in an excavated pit connected to the creek. The sidepools were 2.4 m long, 1.2 m wide, and 0.6 m deep and were divided in half (bays) to offer alternative cover choices to experimental fish (Fig. 1). Sliding screen gates controlled access from the entrance area to the bays and to Dick Creek.

Two sidepools were designed to test, under winter conditions, the preference of juvenile salmonids for an overhanging bank simulating an undisturbed streambank, or a bay barren of cover simulating a damaged streambank. Plywood, cut diagonally and placed 0.4 m above the bay floor provided a "cut-bank" on one side of one bay in the two sidepools (Fig. 1). A dense system of intertwining roots was attached to the bottom of the plywood to serve as cover within the overhanging bank. The adjacent bay was identical except it did not offer overhanging cover (Fig. 1).

The other two sidepools were designed to test the winter preference of juvenile salmonids for clean rubble compared with heavily silted rubble (Fig. 1). A base layer 5 cm deep of gravel and 40 rocks 15–30 cm in diameter were placed in each bay. One hundred liters of sandy-silt was placed in one bay of each sidepool and was sufficient to fill most of the spaces between the rocks. All sidepools were covered with 2.5-cm mesh chicken wire to prevent predation from birds and small mammals.

Experimental Fish

Juvenile coho used in the experiments were obtained from the lowest 200 m of Carnation and Dick creeks by seining in September and electrofishing in January. Juvenile cutthroat were electrofished from a resident population in Ritherdon Creek (an adjacent watershed) in September, January, and February. Prior to January, all fish were held in 1-m² screened pens in Dick Creek until used in the trials. After this time all fish were kept in two 230-liter aquaria supplied with a constant source of Dick Creek water. While held in the pens and aquaria, fish were fed Oregon Moist Pellet; ration was adjusted for water temperature and fish size.

Naive coho were used in the October and January trials; in the November, December, February, and March trials a few "experienced" fish were used. Few fish were used in more than two trials, and a minimum of 2 wk was allowed before any fish was reused. The experimental fish used in the cutthroat trials were predominantly naive individuals. The range and mean fork lengths of all fish used in the experiments are given in Table 1. Scale readings indicated that all experimental fish were age 0 except for 10 small yearling cutthroat.

The number of coho used in the trials was changed from 40 (14/m²) to 20 (7/m²) fish per sidepool following the third trial whereas 20 cutthroat were used in all trout trials (Table 1). Information in the literature and personal observations suggest that the density of experimental fish used in the trials (mainly 7/m²) was high but not unreasonable for overwintering coho and trout. For example, Hartman (1965) reported up to 7.3 coho/m² during the winter and we found coho densities of 5.4/m² in a 10-m² sidepool of Carnation Creek in January. Winter aggregations of up to 75 coho within several square meters were observed by us while snorkeling in Carnation Creek. Density of trout aggregations during the winter is lower than those of coho. Hartman (1965) reported maximum densities of about one steelhead per square meter, and Lindroth (1955a) reported brown trout (*S. trutta*) densities of three to four fish per meter of stream bank. We collected 10 cutthroat of several ages by electrofishing a sidepool of about 2 m² in Ritherdon Creek (five fish per square meter) in midwinter.

Trial Procedure

Each trial took 6 days. On the first day of each trial, experimental fish were placed in the sidepool entrance areas at 0830; from 0930 of day 1 to 0930

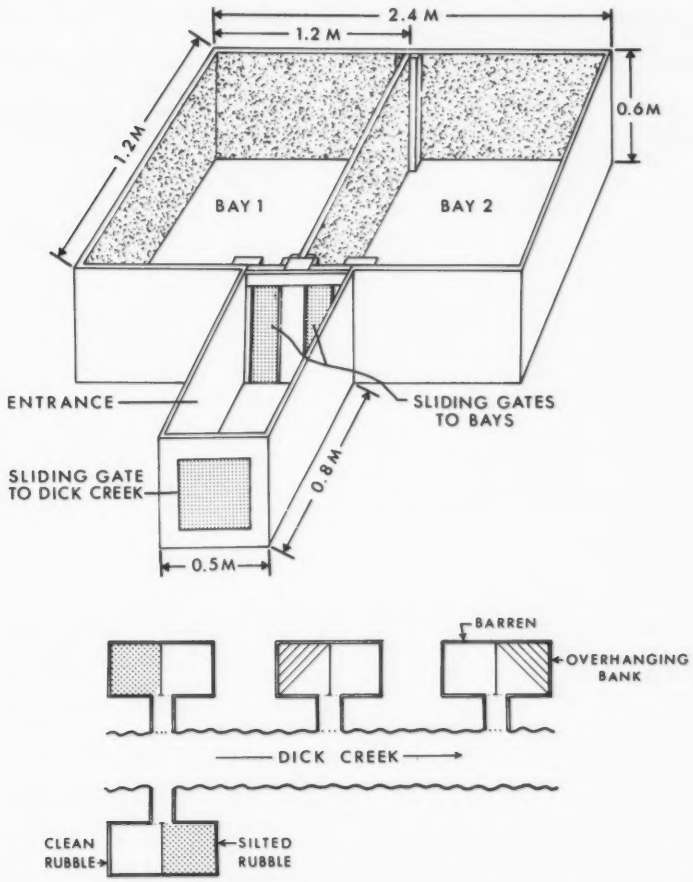

FIG. 1. Design and orientation of sidepools.

of day 2 they were allowed free access to either bay but not to Dick Creek. At 0930 of day 2 the gate to each bay was closed, and in the case of the clean-silted rubble sidepools, the rocks were lifted. (About 24 h were required between removing the rubble and electrofishing to allow the water to clear.) On the morning of day 3 the four sidepools were electrofished and all fish found in each bay of the sidepools were anethesized, counted, and measured to the nearest millimeter. The rubble was replaced in the sidepools and the fish were returned to their respective bays. This ended the first half of each trial (obligatory residence).

At 0930 of day 4, all gates were opened allowing the fish free access into Dick Creek for a 24-h period. Then at 0930 of day 5 the gates were closed and the rubble lifted. On day 6 the number and size of fish remaining in each bay was determined by electrofishing. This ended the second half of each trial (voluntary residence).

Eight preliminary time trials carried out in October for up to 72 h indicated that 24 h was a reasonable period to allow experimental fish to choose between the two bays and between the sidepool and Dick Creek.

EXPERIMENTAL CONDITIONS

Water temperatures were obtained from a Ryan thermograph in Dick Creek and were checked frequently with thermometer readings (Table 1). Temperature was infrequently checked in the sidepools and no differences from the stream temperature were noted. Water level was read from a staff gauge located in one of the sidepools (Table 1).

A total of 12 trials was run between November and

TABLE 1. Date, water temperatures, water depth, number of fish per sidepool, mean fork length, and length range for each trial.

Species	Date	Water temp (*C*)	Water depth (*cm*)	Fish per pool	Mean FL (*mm*)	Range (*mm*)
Coho (Preliminary trials)	Oct. 16 to Oct. 28	6.0–8.0	10–13	40	45.2	37–67
Coho	Nov. 7–12	8.0–9.0	20–34	40	45.9	36–66
"	Dec. 2–7	2.5–5.0	15–17	40	50.5	39–65
"	Jan. 14–19	2.8–5.5	27–47	40	51.2	39–70
"	Feb. 2–7	2.9–4.4	17–22	20	51.8	39–67
"	Feb. 16–21	4.1–4.6	12–22	20	54.2	39–73
"	Mar. 6–11	5.0–6.0	15–26	20	53.2	43–73
"	Mar. 12–17	5.1–6.1	17–22	20	59.1	45–76
"	Mar. 19–24	5.0–5.7	18–32	20	58.4	41–81
Cutthroat	Nov. 18–23	4.5–5.6	12–13	20	60.8	41–75
"	Jan. 26–31	3.5–5.4	17–27	20	63.9	49–91
"	Feb. 22–27	3.5–4.9	13–15	20	63.4	45–91
"	Mar. 1–6	5.8	20–30	20	62.6	45–92

March, with eight using coho and four cutthroat. Stream conditions were variable; therefore, the trials were conducted so that moderate to high and low streamflows were about equally represented.

The coho trials were conducted over a wider range of temperatures than the cutthroat trials. Because stream observations in Carnation Creek indicated that coho were more active above 5 C than below (Bustard and Narver 1975), results of coho preferences at water temperatures above and below 5 C have been separated. We were unable to examine the effects of different temperatures on cutthroat cover preference as all of the trials were at less than 6 C.

Results

Observations of the fish in the sidepools during winter indicated that when allowed access to both bays, groups of fish initially wandered in and out of both sides. For this reason, their choice of bays after 24 h may be considered a "preference." Electrofishing was effective in recovering 98% of all fish used in the trials. Overall mortality resulting from electrofishing, anesthetic, handling, and other causes was 2% in the 12 trials.

COHO

Juvenile coho demonstrated a preference for the bays offering overhanging bank cover as opposed to those without bank cover (Fig. 2). Only the first of the four trials did not indicate this preference, but it was November 7–12, when water temepratures were as high as 9 C, the highest of all trials (Table 1). When given the option of either remaining in the sidepools or of moving into Dick Creek (phase two of the trials), a greater percentage of coho in the four trials remained in the sidepools at lower water temperatures than higher. In both temperature regimes, only 3–4% of the total number of coho originally in the sidepools remained in the barren side as compared to 48–70% in the overhanging bank side.

Coho preferred bays offering clean rubble over bays offering silted rubble, particularly at lower water temperatures (Fig. 2). When given the option of either remaining in the sidepools or of moving into Dick Creek, more coho remained when temperatures were below 5 C than when above 5 C. In both temperature regimes, a greater percentage of the total number of coho originally in the sidepools remained in the bays offering clean rubble than in the silted rubble. At warmer temperatures, the preference was not as obvious, but was still significant (t-test, $P < .01$).

Fewer coho remained voluntarily in the bays offering rubble cover (42% at 2–5 C and 18% at 5–9 C) than in those providing overhanging bank cover (73% at 2–5 C and 52% at 5–9 C).

CUTTHROAT

Juvenile cutthroat trout showed a strong prefer-

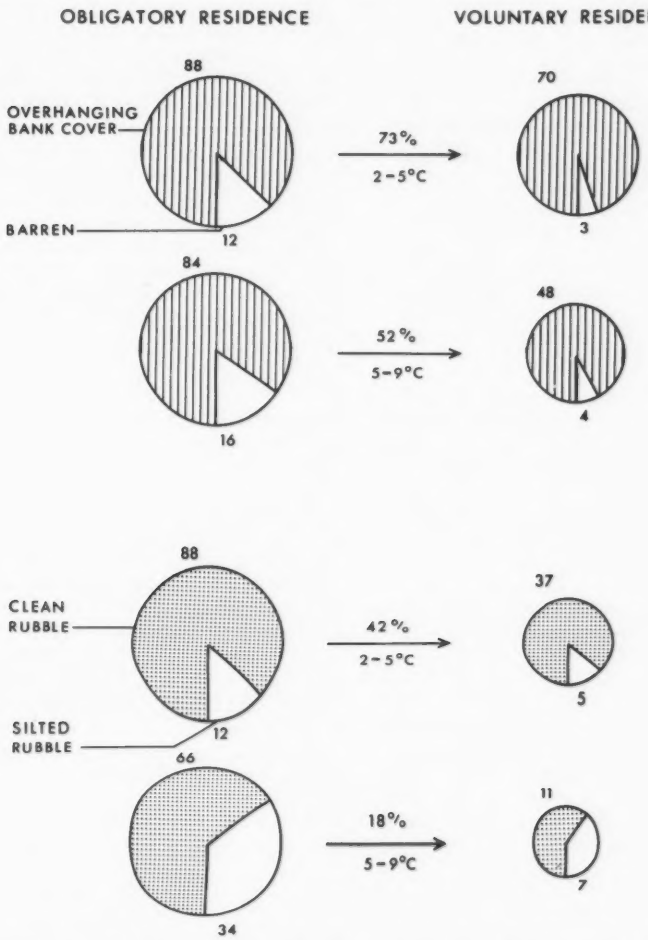

FIG. 2. The percentage of coho salmon (*Oncorhynchus kisutch*) choosing alternative cover types in the sidepools when access to Dick Creek was not permitted (obligatory residency) and permitted (voluntary residency). The percentage of coho remaining in the sidepools after having access to the creek is indicated above the arrows. The figures are for two temperature ranges (indicated below the arrows), and each figure represents a combination of data from four trials each replicated.

ence for bays offering overhanging bank cover as opposed to no cover (Fig. 3). In two of the four trials, all of the experimental fish chose the overhanging bank side. Of the 46% choosing to remain when given the option of moving into Dick Creek, only 1% chose the barren side.

Cutthroat strongly preferred bays offering clean rubble to those offering silted rubble (Fig. 3). Nearly half of the original cutthroat in the sidepools remained when allowed access to Dick Creek, and all fish but one were found in the clean rubble bays.

Discussion

The sidepool experiments effectively demonstrated juvenile coho and cutthroat preference for

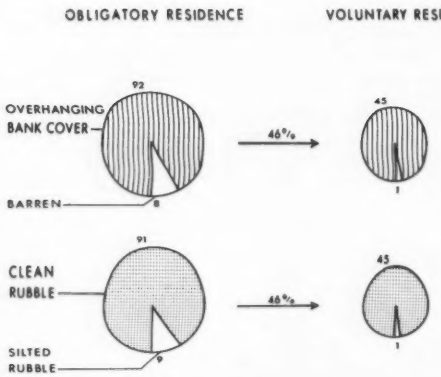

FIG. 3. The percentage of cutthroat trout (*Salmo clarki*) choosing alternative cover types in the sidepools when access to Dick Creek was not permitted (obligatory residence) and permitted (voluntary residence). The percentage of cutthroat remaining in the sidepools after having access to the creek is indicated above the arrows. Each figure represents a combination of data from four trials each replicated.

winter cover consisting of overhanging banks over no cover and clean rubble over silted rubble. For coho this was most evident at lowest water temperatures. The low numbers of fish initially choosing and remaining in the sidepools containing no bank cover or silted rubble at low temperatures indicated that these types of habitat did not provide suitable winter cover. These results agree with our underwater observations in Carnation Creek, where nearly all fish were found closely associated with cover (Bustard and Narver 1975). In the trials a higher percentage of coho voluntarily utilized bank cover than rubble cover, whereas cutthroat used both bank cover and rubble equally (Fig. 2 and 3). These results also agree with our underwater and electrofishing observations in the stream. That coho do not utilize rubble as overwinter cover was suggested by Ruggles (1966) when he added concrete blocks to a gravel stream channel. He found the channel's capacity to hold young coho over the winter was actually reduced.

Studies elsewhere have demonstrated the importance of shelter areas to fish survival but few have involved research during winter conditions. For example, McCrimmon (1954) showed clearly that heavily silted rubble reduced Atlantic salmon (*S. salar*) fingerling survival in the fall by eliminating escape cover. Hunt (1971) showed that by adding overhanging bank cover and increasing the number of pools, overwinter survival of brook trout was nearly doubled. Removal of overhanging vegetation and banks resulted in a decrease in the trout population of a small Montana stream (Boussu 1954). By increasing the number of hiding places in a small stream, Saunders and Smith (1962) nearly doubled within 1 yr the population of age I and older brook trout. They also showed reduced trout populations as a result of destruction of hiding places by heavy siltation (Saunders and Smith 1964).

The experiments with the sidepools, along with underwater observations (Bustard and Narver 1975) led us to suggest some consequences of stream damage. Logging operations, channel clearance and straightening, and road construction activities adjacent to stream areas have been shown to result in bank damage (Anderson 1971; Burns 1972; Narver 1972). We infer that such activities may alter sections of streams from our experimental "bank cover" to the "no bank cover."

Increased sediment loads in streams following logging operations have been well documented and mainly attributed to poor road designs (Jeffrey 1968; Fredriksen 1970; Megahan and Kidd 1972), slashburning (Brown and Krygier 1971), and erosion of disturbed streambanks (Anderson 1971; Burns 1972). Severe siltation can reduce salmonid populations as noted above. Our experimental "clean rubble" may be altered to "silted rubble" by siltation.

The sidepool experiments permitted volitional residence of fish. As nearly all fish left the bays with no bank cover or silted rubble, one would suspect these fish would avoid stream sections similarly lacking in cover. Although the trials do not indicate whether or not fish would survive in areas lacking cover, we suspect survival would be low. When electrofishing or snorkeling in Carnation Creek we found very few fish in the absence of cover (Bustard and Narver 1975).

This study provides additional evidence of the importance of maintaining the integrity of streambank areas during logging by keeping operations away from the stream. Also suggested is the need to keep erosion and resulting silt deposition to a minimum.

Acknowledgments

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Movements and Growth of Arctic Grayling (*Thymallus arcticus*) and Juvenile Arctic Char (*Salvelinus alpinus*) in a Small Arctic Stream, Alaska

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CRAIG, P. C., AND V. A. POULIN. 1975. Movements and growth of Arctic grayling (*Thymallus arcticus*) and juvenile Arctic char (*Salvelinus alpinus*) in a small arctic stream, Alaska. J. Fish. Res. Board Can. 32: 689–697.

Fish movements were monitored in a small arctic stream that drains into the Kavik River near Prudhoe Bay, Alaska. The stream, which flows only during summer, served as a spawning and nursery area for Arctic grayling (*Thymallus arcticus*). Adult grayling entered the stream shortly after breakup and left again after spawning. Juvenile grayling also entered soon after breakup and many remained throughout the summer. Grayling fry emerged from the gravel in late June or early July and remained until freeze-up (mid-September). Small numbers of Arctic char (*Salvelinus alpinus*) juveniles also entered the stream to feed.

The growth rate of grayling in the study stream was among the fastest recorded for arctic populations. Otolith-based age determinations were preferred as scales tended to underestimate the ages of old fish. Maximum observed age was 16 and age at sexual maturity ranged from 5 to 8.

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Nous avons suivi les déplacements de poissons dans un petit cours d'eau arctique tributaire de la rivière Kavik, près de la baie Prudhoe, Alaska. Le cours d'eau, qui ne coule qu'en été, est utilisé par l'ombre arctique (*Thymallus arcticus*) pour la fraie et l'alevinage. Les ombres arctiques pénètrent dans le cours d'eau peu après la débâcle et le quittent après la fraie. Les jeunes ombres arctiques pénètrent eux aussi dans le cours d'eau peu après la débâcle, et plusieurs y séjournent tout l'été. Les alevins émergent du gravier tard en juin ou tôt en juillet et demeurent dans le cours d'eau jusqu'au gel (mi-septembre). Un petit nombre de jeunes ombres arctiques (*Salvelinus alpinus*) pénètrent également dans le cours d'eau pour s'y nourrir.

Le rythme de croissance de l'ombre arctique dans le cours d'eau sous étude est un des plus rapides signalés chez des populations arctiques. On a préféré les otolithes aux écailles pour déterminer l'âge, parce que les écailles ont tendance à sous-estimer l'âge des vieux poissons. L'âge maximal observé est de 16 ans et l'âge de la maturité sexuelle varie entre 5 et 8 ans.

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ALTHOUGH the Arctic grayling (*Thymallus arcticus*) is a prized sport fish and a common inhabitant of arctic streams (McPhail and Lindsey 1970; Scott and Crossman 1973), there have been few published studies of this species in arctic regions. Movement data are particularly scarce (McCart et al. 1972). Studies in more southern streams suggest complex and varying patterns of movements for this species (e.g. Reed 1964; MacPhee and Watts 1973; K. Chang-Kue unpublished data), and therefore a comparative study was conducted in a habitat that is ice-locked for much of the year.

The study area is a small tundra stream on Alaska's Arctic Coastal Plain. Grayling made opportunistic use of the stream as a spawning and nursery area during the short arctic summer, and a few juvenile Arctic char (*Salvelinus alpinus*) entered the stream to feed. Their movements and growth are described and compared to other arctic and subarctic populations. As many similar streams in the north are likely to be affected by roadways, pipelines, and increased human activity in general, it is important that fish utilization of these streams be investigated.

Methods

During the summers of 1972 and 1973, fish were captured in Weir Creek by gillnet, seine, and angling for life history analysis. In 1973, we maintained a

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fish weir to study fish movements. The weir, located 0.8 km upstream of the stream mouth, completely blocked the stream with a small mesh (1.27 cm) wire screen that was placed diagonally across the stream to guide fish into the holding traps at either end. Holding traps (0.63-cm screen mesh) were checked daily. The weir was established on June 15, approximately 2 wk after flow began and was permanently removed September 26, shortly before flow ceased. It was inoperable during five floodwater periods for a total of 27 days (26%) inoperation. Grayling were classified as adults (>300 mm) or juveniles (<300 mm) on the basis of previous dissection data.

To study fish movements prior to operation of the weir, gillnets were set at stations in Weir Creek and the Kavik River. Between May 31 and June 15, 1973, 35 gillnets were set, each for approximately 24 h. In addition, we tagged 107 Weir Creek grayling with Floy dart tags to study their movements.

Laboratory analysis included determination of fork length to the nearest millimeter, weight to the nearest 0.1 g, sex, and maturity. Otoliths and scales were aged under a binocular microscope (otoliths) or scale enlarger. Criteria for identification of annuli on otoliths were similar to those of Nordeng (1961).

Water velocities for discharge estimates were determined with a Gurley Pygmy Current Meter. Turbidities (ppm SiO_2) were measured with a Hellige Turbidimeter (model TR 3000).

Description of the Study Area

The study stream (69°41′15″N, 147°07′30″W), unofficially designated Weir Creek, originates in the northern foothills of the Brooks Mountain Range and flows approximately 39 km before entering the Kavik River (Fig. 1). It drains 155 km^2 of tundra-covered hills and is similar to the tundra streams described by Craig and McCart (1975). There are no spring sources on Weir Creek and flow is derived from snowmelt and rainfall. The stream is typically 5–10 m wide and 25–50 cm deep, although it spreads across the entire floodplain (10–25 m) during spates.

Spring breakup occurred swiftly in Weir Creek. The stream was frozen on May 29, 1973, but 2 days later, floodwaters (17 m^3/s) overflowed high banks and spread across stream meanders in some areas. Summer discharge generally ranged from 1 to 6 m^3/s. Flow ceased about October 15. The open water period extended approximately 4.5 mo in 1973, 1 mo longer than in the previous year (May 27–September 13, 1972).

Turbidity levels during breakup reached a maximum (30 ppm SiO_2) on June 8. Thereafter, turbidity was low except after heavy rainstorms when levels increased from $\leqslant$5 to 20 ppm SiO_2. Weir Creek was consistently less turbid than the Kavik River. During spring breakup, maximum turbidity levels in Weir Creek were 54% lower than those recorded in the Kavik River and levels during spates were approximately 35% lower.

Diel and seasonal water temperatures in Weir Creek were highly variable and responded rapidly to changes in air temperatures. The maximum water temperature recorded was 19 C (July 5) and the lowest summer temperatures were 1.7 C (July 14) and 1.1 C (August 7). Between June 15 and August 15, maximum daily water temperatures frequently (30%) reached 15 C or greater. Diel variation commonly ranged between 3 and 6 C, although differences of 10 C were recorded.

Distribution and Movements of Fish

Grayling, Arctic char and ninespine sticklebacks (*Pungitius pungitius*) inhabit the Kavik River, but few of the latter are present. Within the drainage, grayling and char distributions overlap, although grayling are most abundant in tributaries and lower reaches of the Kavik, whereas char extend further upstream and are more abundant in the mainstream than tributaries. This spatial distinction is emphasized more clearly in the distribution of fry and the location of spawning habitats. Grayling spawn in tundra tributaries, whereas char spawn in a spring-fed region in the upper Kavik River.

Within Weir Creek, both grayling and char were collected as far as 27 km upstream but were most common in the lower reaches. During the study period, 18,123 fish were enumerated at the weir in this stream. Of this total, 0.1% were adult grayling, 11.9% juvenile grayling, 86.8% grayling fry, and 1.2% Arctic char. The size distributions of all fish (excluding grayling fry) caught are shown in Fig. 2. The movements of these fish are discussed in the following sections.

Grayling Adults

Distribution of grayling adults (as well as juveniles) is extremely restricted during the long arctic winter when most of the drainage is frozen solid. Only two spring-fed overwintering areas were located (Fig. 1). Grayling leave these areas to spawn shortly after spring thawing and flooding begin in late May and early June. Gillnet data show that grayling adults entered Weir Creek around June 7, 1 wk after flow began in this tributary, and spawned from June 11 to 18.

Physical conditions in Weir Creek changed rapidly during the spawning period. Maximum water temperatures increased from 3.9 C (June 11) to 16.7 C (June 16). Discharge levels dropped from an estimated 17 m^3/s (June 11) to 1 m^3/s (June 20). Similarly, turbidity declined to low levels.

Adults swiftly departed Weir Creek after spawn-

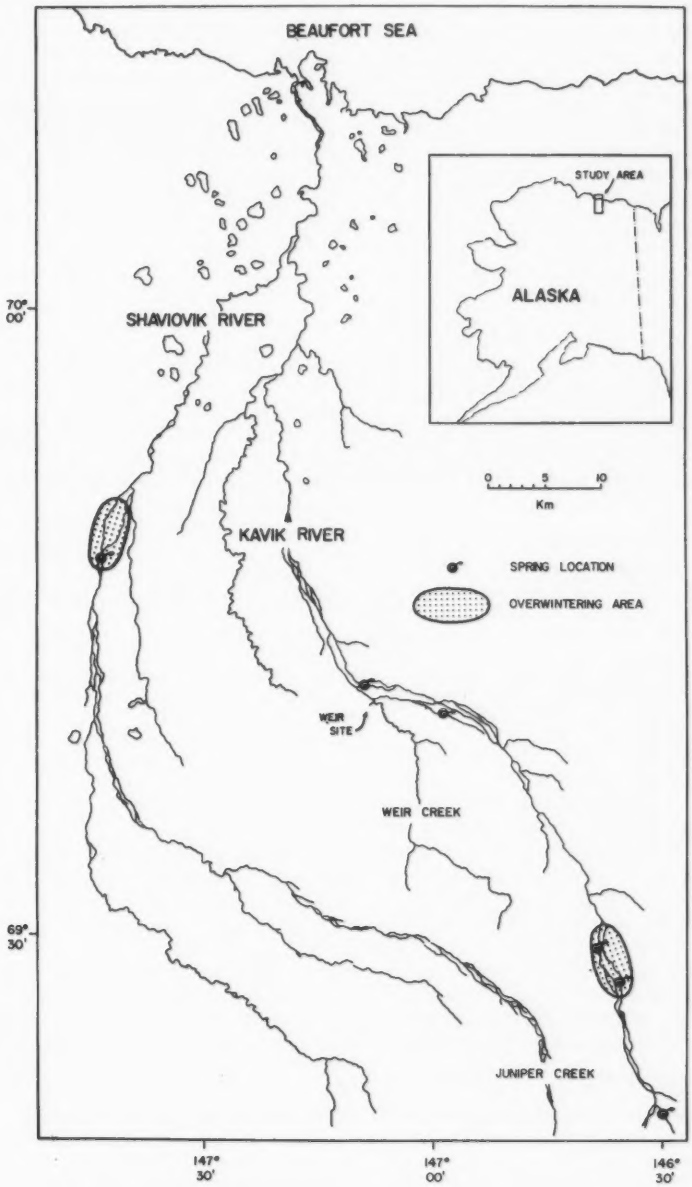

FIG. 1. Shaviovik River drainage. Weir Creek, overwintering areas, and the location of perennial springs are indicated.

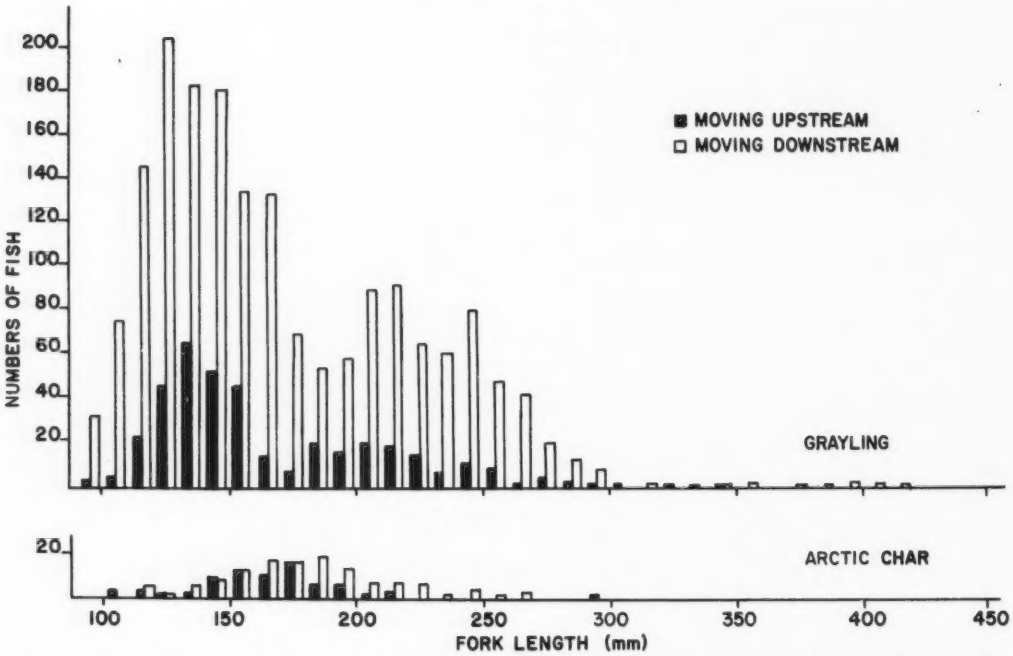

FIG. 2. Length frequency of all Arctic grayling (*Thymallus arcticus*) (excluding fry) and Arctic char (*Salvelinus alpinus*) moving upstream and downstream through the weir at Weir Creek, 1973.

ing as none was caught in the weir after July 3. Some adults probably remained in the stream and emigrated during summer floods when the weir was out. Some additional information about the movements of adult grayling is provided by the recapture of four fish that were tagged in Weir Creek. One tagged grayling left Weir Creek soon after spawning and was recaptured nearby in the Kavik River. Two other grayling were recaptured 1 yr later in or near Weir Creek, suggesting that these fish may return to the same stream to spawn.

The fourth tagged fish was recaptured while overwintering in the Shaviovik River, 85 km distant from Weir Creek (Fig. 1). It appears that most grayling in the Kavik drainage overwinter at this location as large numbers of overwintering grayling (and Arctic char) were observed there on April 12, 1973. The only other known overwintering area in the drainage is in the upper Kavik River, 16 km upstream of Weir Creek. This site was repeatedly sampled throughout the summer and fall, and although char were abundant, few grayling were caught.

JUVENILE GRAYLING

Juvenile fish also entered Weir Creek in large numbers soon after breakup. Some juveniles remained in Weir Creek throughout the season, but a large proportion, especially the larger juveniles, emigrated 2–3 wk after the adults had departed. Stream flow was low at this time and areas suitable for maintaining territories were possibly reduced (Vascotto 1970). Reed (1964) also noted that low water levels and warmwater temperatures may influence the movements of juvenile grayling. An unusually large number of juveniles (259) emigrated on a single day (July 12), but this behavior did not appear to be correlated with any change in stream conditions.

By mid-July, small juveniles (<200 mm) and newly emerged fry remained in Weir Creek. There was a general emigration of juveniles, but activity increased in September as water temperatures cooled. Emigration peaked September 7–11, when an average of 85 juveniles per day were caught. Numbers dropped to one juvenile per day

(September 21–26) prior to removal of the weir. Freeze-up occurred approximately 1–2 wk later.

There appeared to be no correlation between several physical parameters and numbers of juveniles moving through the weir. For the days the weir was in operation, no significant correlation was found with maximum daily water temperatures ($r = 0.0$, $n = 65$, $P > 0.1$), minimum daily water temperatures ($r = 0.0$, $n = 66$, $P > 0.1$), or water height during the midsummer period, July 1–August 17 ($r = 0.24$, $n = 37$, $P > 0.1$).

Grayling Fry

Grayling fry were the most abundant group in Weir Creek, accounting for 86.8% of all fish passing through the weir. Fry emerged from the gravel in late June or the first week of July and remained in the stream throughout the short summer. During September, 15,729 fry passed through the weir. At the peak of emigration, September 13–21, fry numbers were often greater than 1,000/day with a maximum of 2,200 fry on September 20. Numbers quickly declined and only 10 fry were taken the last day the weir was in operation.

Arctic Char

After spring breakup, anadromous char emigrate to the ocean. Most presmolts remain in the main river, although some enter the tundra tributaries. In Weir Creek, for example, only 216 char were caught (1.2% of all fish enumerated at the weir) and these were almost exclusively juveniles. During the open water period, there was a small degree of char movement into and out of Weir Creek.

In fall and early winter when Weir Creek and other tributaries are frozen solid, anadromous char move onto their spawning grounds in the Kavik and Shaviovik rivers (Fig. 1). These locations also serve as the overwintering areas for char (as well as grayling) from September to May.

Grayling Life History

Aging Technique

Most researchers traditionally use the scale method to age grayling, but several authors indicate that scale-based ages of northern populations may underestimate grayling ages for two reasons: a) the first annulus may be missing, and b) there is difficulty in identifying annuli on the scales of older fish because the small amount of yearly growth results in little incremental scale growth (Kruse 1958; Shallock 1965; Bishop 1967; McCart et al. 1972; de Bruyn and McCart 1974).

Therefore, grayling ages were determined by both scale and otolith methods. Both methods gave similar ages through age 8, but the scale method appeared to underestimate the ages of older fish (Fig. 3). Maximum-scale determined age was 11, compared to 16 for otoliths. Thus, a consequence of using scale-based ages for northern populations may be a somewhat exaggerated growth rate (Table 1). For this reason, otolith-based ages were used in this study.

Data were combined for Weir Creek and Kavik River locations and also for males and females because significant differences in the length–age relationships could not be demonstrated. These data do not reflect size or age frequencies of grayling in the study area as fish were selectively sampled to provide similar representation within size-classes.

Growth of grayling in the study area was moderately slow. Fry reached a mean fork length of 60 mm (range 44–70 mm) by the end of their first year. Thereafter, grayling grew approximately 40 mm/year through age 7, attaining most of their size by this age (Table 1). Later growth was slower and few fish reached more than 380 mm fork length.

Age at maturity was similar for males and females. The youngest mature fish was age 5, and all fish were mature by age 8. All mature fish exceeded 295 mm (11.6 in.) fork length ($n = 92$). Once mature, grayling apparently spawn every year. All fish beyond age 8 contained sex products that would mature the following spring.

To obtain a weight–length relationship, three fish were randomly selected for each 10-mm increment in fork length (range 40–440 mm). The following relationship ($r = 0.99$) was obtained for 110 grayling from Weir Creek and the Kavik River:

$$\log_{10} \text{weight (g)} = 3.151 \log_{10} \text{length (mm)} - 5.346$$

The largest grayling caught measured 435 mm (17.1 inches) and weighed 886.2 g (2 lb).

Arctic Char Life History

A relatively small number of Arctic char utilized Weir Creek as a rearing area. Most of these fish were small, juvenile char (Fig. 2) between ages 1 and 3. Only 6% of the sample ($n = 64$) were mature and these were small, residual (stream-

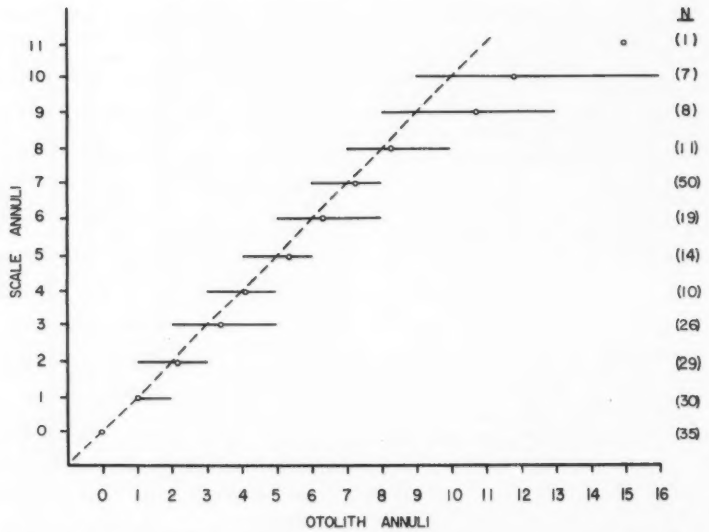

FIG. 3. Comparison of grayling ages derived by the scale and otolith methods for the same sample of 240 fish. The dashed line indicates a 1:1 relationship between the two techniques. For each scale-based age, the mean (dot) and range (longitudinal bars) of otolith-based ages for the same fish are given. N = the number of fish in each scale-based age-class.

TABLE 1. Observed age–length relationships derived by otolith and scale methods of Arctic grayling (*Thymallus arcticus*) from Weir Creek and the Kavik River. N = number of fish in sample; SD = standard deviation.

	Otolith method				Scale method			
		FL (*mm*)				FL (*mm*)		
Age	N	mean	(range)	SD	N	mean	(range)	SD
1	48	94.3	(66–122)	10.2	46	97.9	(84–125)	11.4
2	25	133.3	(103–155)	13.2	30	139.9	(91–182)	19.1
3	35	182.6	(142–238)	23.6	27	191.5	(133–238)	25.1
4	24	208.0	(133–252)	30.8	11	234.5	(205–267)	19.6
5	21	258.0	(169–326)	41.6	14	289.8	(227–336)	35.6
6	17	312.3	(227–338)	26.7	20	319.2	(245–348)	27.7
7	40	335.6	(300–367)	16.1	38	340.7	(300–379)	17.9
8	20	354.7	(332–382)	15.3	13	366.5	(332–403)	17.7
9	4	370.5	(352–385)	12.0	8	373.3	(350–398)	17.4
10	5	369.8	(351–400)	16.9	6	391.0	(370–418)	17.8
11	5	382.2	(370–401)	10.2	1	398.0		
12	3	388.0	(385–392)	2.9				
13	2	405.0	(398–412)	7.0				
14	1	373.0						
15	1	398.0						
16	1	418.0						
Totals	252				214			

resident) males, ages 4 and 5. This pattern of maturity appears similar to that described for immature and residual members of other anadromous populations of North Slope char (McCart et al. 1972; Bain 1974; Glova and McCart 1974).

Discussion

Growth of Weir Creek grayling fits midway between previously studied populations exhibiting the fastest growth (e.g. Nelson 1954; Bishop 1967), and slowest growth (e.g. McCart et al. 1972; de Bruyn and McCart 1974). As a generalization, northern populations of grayling tend to grow slower, live longer, and reach smaller maximum sizes than those in the south. There is some difficulty in making such comparisons, however, due to the different aging techniques used by various authors.

If only populations in Beaufort Sea drainages are compared (McCart et al. 1972; de Bruyn and McCart 1974), the growth of grayling in Weir Creek is among the fastest. In addition, they attained a greater maximum size (1.5–6.5 cm longer) than other North Slope grayling populations. Longevities of these northern grayling vary from population to population, but few fish, in general, live longer than age 16. The oldest grayling caught in these populations have been age 15 (Trout Lake), age 16 (Weir Creek, Lake 100), age 19 (Kuparuk River), age 20 (Happy Valley Creek), and age 22 (Firth River). Additional data are available for the Colville River (Kogl 1972) and combined grayling samples from North Slope waters in Alaska (Roguski and Winslow 1970) and the Yukon Territory (Bryan et al. 1973). Maximum scale-based ages from these sources range from age 8 to 11.

The pattern of grayling movements in Weir Creek appears typical of grayling in small tributary streams in Alaska and northern Canada: a) Happy Valley Creek, a 25–km tundra stream (McCart et al. 1972); b) McManus Creek, a 32-km stream in interior Alaska (Vascotto 1970); c) a 2.6-km tributary of Fielding Lake (Warner 1957); d) Three Day Lake drainage (K. Chang-Kue unpublished data); and e) Polar Grove Creek, an 8-km tributary of the Copper River (MacPhee and Watts 1973). In Popular Creek, however, the timing of movements differed slightly, reflecting the longer open water season (April 21–October 21, 1973). A schematic diagram of these movements is shown in Fig. 4. In larger streams, an important variation of this pattern is the length of time that the mature fish remain in the spawning stream. Some may stay until midsummer or autumn (Reed 1964; Tripp and McCart 1974).

Utilization of small streams by grayling and the pattern of grayling movements within these streams appear to result in a number of advantages for these fish, especially for juveniles and fry. Despite the short growing season, the warm waters (up to 17 C) and the abundance of food items in tundra streams (Craig and McCart 1975) provide favorable conditions for growth. Grayling fry in Weir Creek are able to equal the growth of Arctic char fry, although char may emerge several weeks earlier and at a larger size. (McCart et al. 1972). This temporal and spatial pattern may also reduce competition between young grayling and other species or the older age-groups of their own species. Small grayling may compete with large grayling for the same food items (Vascotto 1970), and as observed in this study (unpublished data), the diet of grayling is similar to that of juvenile Arctic char.

As more streams in a drainage system are studied, different patterns of movements and utilization may become apparent. There is evidence, for example, that a) some grayling return annually to a particular stream to spawn, and b) some tributaries serve as spawning streams and rearing areas for fry, whereas other tributaries, mainstreams, or lakes are used by immatures and adults as summer feeding areas (Warner 1957; Nagata and Van Whye 1963; Van Whye 1964; Reed 1964; Shallock 1965; Tack 1971; Tripp and McCart 1974; K. Chang-Kue unpublished data).

In the context of fisheries management, the complexity of movement patterns demonstrates the interrelationship between parts of a drainage and stresses the importance for studies that follow grayling movements through all stages of its life cycle. The Weir Creek study, for example, identifies a particularly vulnerable stage for grayling when the entire population is concentrated in small spring-fed overwintering sites and confined to these areas throughout the 7–8-mo arctic winter.

Life history analysis also has management implications for northern grayling populations. First, scale-based ages may underestimate the ages of older grayling, producing a somewhat exaggerated growth rate for northern populations. Second, because northern fish tend to grow slower, mature later, and live longer than southern populations, it is conceivable that the northern populations may be more susceptible to certain adverse effects of human interference. Falk and Gillman (1974) present convincing evidence that, even in a water body as large as Great Slave Lake, sport fishing pressure over a 7-yr period has caused a reduction in both the average size and age of angled gray-

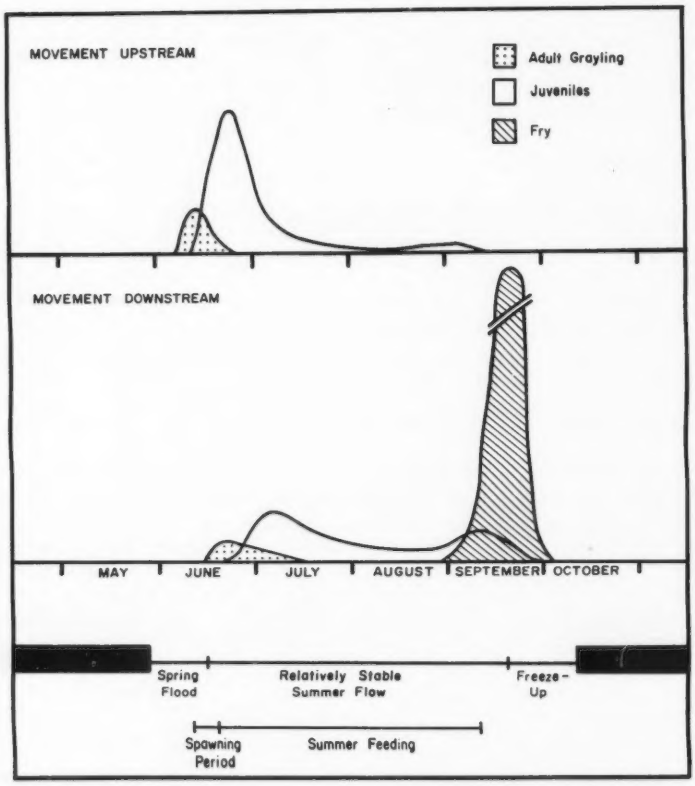

FIG. 4. Schematic diagram of grayling movements in small northern streams.

ling, with the new modal age approaching the age at first sexual maturity.

Acknowledgments

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Simple and Rapid Technique for Repeated Blood Sampling in Rainbow Trout (*Salmo gairdneri*)

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LIED, E., J. GJERDE, AND O. R. BRAEKKAN. 1975. Simple and rapid technique for repeated blood sampling in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 699–701.

A method for repeated blood sampling from small as well as large fish by puncturing the duct of Cuvier is described. The method is simple and makes possible rapid sampling of relatively large volumes of blood. The method is thought to be less stressing for the fish than earlier methods described. Application of the technique to 20- and 300-g trout resulted in no deaths or apparent damage during a 1-mo observation period.

LIED, E., J. GJERDE, AND O. R. BRAEKKAN. 1975. Simple and rapid technique for repeated blood sampling in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 699–701.

Les auteurs décrivent une méthode de prélèvements de sang répétés, par ponction du conduit de Cuvier, sur des poissons de petite, aussi bien que de grande taille. La méthode est simple et permet de prélever rapidement des volumes de sang relativement grands. On croit que cette méthode produit chez les poissons un choc moins fort que les méthodes déjà décrites. L'application de la technique à des truites de 20 et 300 g n'a provoqué aucune mortalité ou dommage apparent durant une période d'observation de 1 mois.

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IN physiological and nutritional studies of fish analysis of blood is often required. Blood sampling can be carried out simply by cutting the throat, severing the caudal peduncle, or by more refined procedures such as cardiac puncture, puncture of the caudal artery or vein (Itazawa 1957), repeated sampling by puncturing the dorsal aorta (Schiffman 1959), and prolonged sampling in free swimming fish by cannulating the dorsal aorta (Smith and Bell 1964).

In a nutritional study on rainbow trout it was necessary to sample nonlethally adequate amounts of blood from small and large fish with a minimum of stress to the fish. Since none of the existing procedures were satisfactory for our purpose, a technique for rapid and repeated blood sampling was developed and applied to rainbow trout.

Materials and methods — Rainbow trout (*Salmo gairdneri*) of 12–30 cm (20–500 g) were used as experimental animals. The fish were obtained from cultured stocks, kept in seawater tanks during the experimental period, and fed once a day. Blood sampling was always carried out before, or 4–5 h after feeding to avoid regurgitation.

Anesthesia was carried out by transferring fish individually to a tank with sea water containing 2.5 ml saturated ethanolic solution of benzocain per 10 liters (Wedemeyer 1970). A fish was anesthetized to a point equivalent to Stage III, Plan 2 according to the classification of the stages of anesthesia of fishes (Klontz and Smith 1968) and put on a table. Samples were drawn as quickly as possible. The fish were revived either by transfer into a tank with fast flowing water or simply by putting the fish into the main tank and irrigating the gills through the mouth with a water hose.

For fish above 100 g, 2 or 5 ml disposable syringes fitted with 0.80 × 40 mm disposable needles were most convenient. For fish of 20–100 g, 1 ml tuberculin syringes equipped with 0.50 × 20 mm hypodermic needles were preferred. Heparin was used as an anticoagulant.

Procedure — A rainbow trout is anesthetized and placed on a table with the left or right side upwards. A spatula is inserted under the gill bars of the fourth gill arch and into the fifth gill slit. For

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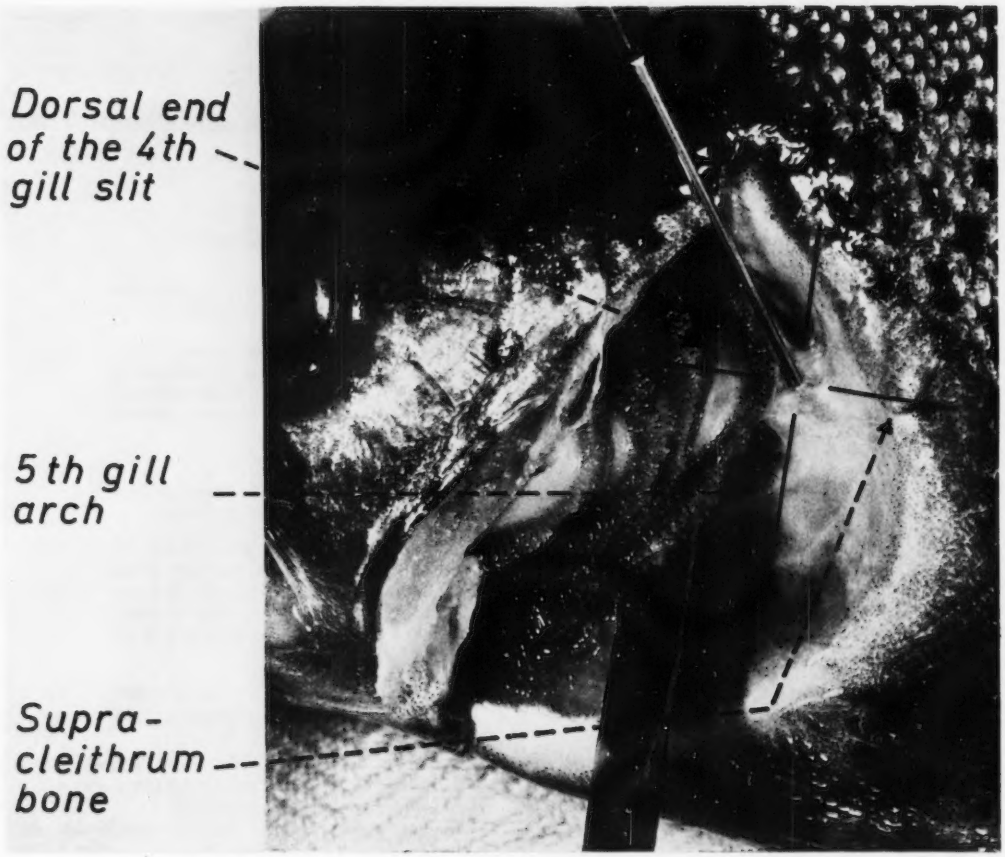

FIG. 1. Sampling of blood from rainbow trout (*Salmo gairdneri*). Operculum and gill bars have been dissected away to illustrate the point of insertion. The fourth gill arch is lifted by a spatula and the needle inserted where an imaginary horizontal line, drawn from the ventral end of the supracleithrum bone to the dorsal end of the fourth gill slit, crosses a vertical line drawn through the dorsal end of the fifth gill arch.

a fish of approximately 300 g, the fourth gill arch should be gently lifted 10–15 mm. An important step in the procedure is finding the exact point of insertion and inserting the needle at the correct angles to penetrate the duct of Cuvier. The entry is made where an imaginary horizontal line, drawn from the ventral end of the supracleithrum bone to the dorsal end of the fourth gill slit, crosses a vertical line drawn through the dorsal end of the fifth gill arch as demonstrated in Fig. 1. Holding the heparinized syringe at an angle of about 60° craniad to a horizontal cranial–caudal plane and about 20° dorsal to a vertical cranial–caudal plane through the fish, the needle is slowly inserted about 5 mm into the tissue while making a slightly reduced pressure in the syringe by gently lifting the piston with the thumb. When the duct of Cuvier is penetrated blood flows into the syringe and perforating the vessel wall at the opposite side of the puncture is avoided. Using the thumb to operate the piston, the desired amount of blood is slowly drawn. Making this operation too fast very often flattens the vein, blocking the flow of blood. If this happens, the needle is withdrawn a couple of millimeters and reinserted a few seconds later. Minor but short-lived bleeding occurred at the puncture site after the needle had been withdrawn.

Sampling unanesthetized fish requires the use of a holder. As such we have used clips made of

wire, lined with foam plastic and formed to fit the individual fish. The fish is placed in the clip, forced to lie still by pressing, and the sampling is carried out through an opening, exposing the operculum and gill apparatus.

When sampling from small fish no modification in technique is required except for the use of different needles and syringes as described previously. Routine samples of 0.15–0.20 ml have been drawn from fish of 20 g. Due to the narrower needles applied, the possibility for blockage of the flow by blood clotting is increased compared with the sampling of larger fish. With practice, a sample equivalent to 1% of the weight of the fish could be drawn in approximately 30 s. To reduce the stress the fish were never out of water for more than 1 min.

The effect of sampling on long-time survival of 20- and 300-g trout was tested by keeping 60 fish of each weight group in aquaria after sampling 0.15–0.20 and 2.5–3.0 ml of blood, respectively, and observing them for 1 mo. None of the fishes died and all seemed to be normally active. However, complete revival of the fish from the anesthesia is most important in preventing deaths.

By macroscopic inspection of a randomly drawn sample of fish 30 min after the puncture of the duct of Cuvier, neither external nor internal bleeding could be seen, and by 1 mo later the marks of insertion had disappeared.

Discussion — Sampling by the technique of puncturing the duct of Cuvier was found to have several advantages which should be considered when choosing methods for collecting blood from fish. In contrast to methods such as cutting the throat or severing the caudal peduncle, samples can be drawn repeatedly from the same fish, and free from tissue fluids. The frequency of sampling without endangering the fish varies with the species. Klontz and Smith (1968) have indicated, however, that blood volumes equivalent to 1% of the body weight, i.e. 25–30% of the blood volume, can be drawn weekly from salmonid fishes.

Since blood sampling seems to be carried out most frequently by cardiac puncture, a comparison with this technique is of special interest. In our opinion routine sampling by puncturing the duct of Cuvier, could be performed much more easily than by cardiac puncture. In addition, internal damage and bleeding caused by cardiac puncture can be considerable. Approximately half an hour after having sampled by the cardiac puncture, autopsy often showed that the pericardium was filled with blood clots that in addition to one or several wounds in the ventricle most likely would interfere with the work of the heart and thereby increase stress on the fish. In contrast, our technique avoids such interfering factors since neither internal bleedings nor external or internal damages were observed.

The duct of Cuvier has previously been used for injections of dye into the vascular system of dogfish followed by sampling from the caudal artery for computations of cardiac output (Murdaugh et al. 1965). We plan to investigate the possibility of combining puncture of the duct of Cuvier with cannulation of the dorsal aorta (Smith and Bell 1964), as a method for studying the excretion of metabolites through the gill.

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Red Crab (*Geryon quinquedens*) and Snow Crab (*Chionoecetes opilio*) Resistance to Infection by the Lobster Pathogen *Aerococcus viridans* (var.) *homari*

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CORNICK, J. W., AND J. E. STEWART. 1975. Red crab (*Geryon quinquedens*) and snow crab (*Chionoecetes opilio*) resistance to infection by the lobster pathogen *Aerococcus viridans* (var.) *homari*. J. Fish. Res. Board Can. 32: 702–706.

Red crabs (*Geryon quinquedens*) were acclimated to water temperatures up to 14 C without excessive mortality. The lobster pathogen *Aerococcus viridans* (var.) *homari* was nonpathogenic for the red crab at 5, 10, and 15 C and for the snow crab (*Chionoecetes opilio*) at 5 C, but could be recultured from each species after 60 and 100 days, respectively, with no loss of virulence for the lobster. The lack of virulence was not due to the inadequacy of the hemolymph as a growth medium. Although the serum of each species contained natural bactericidal and agglutinating principles, only the snow crab agglutinin was active against *A. viridans* (var.) *homari*. The resistance of these crab species is discussed in relation to their potential as reservoirs of infection.

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Les auteurs ont acclimaté des crabes rouges (*Geryon quinquedens*) à des températures d'eau de 14 C sans qu'il se produise de mortalités excessives. L'organisme pathogène du homard, *Aerococcus viridans* (var.) *homari* est non pathogène pour le crabe rouge à 5, 10 et 15 C et pour le crabe des neiges (*Chionoecetes opilio*) à 5 C, mais peut être cultivé de nouveau à partir de chaque espèce après 60 et 100 jours respectivement, sans qu'il y ait diminution de virulence à l'endroit du homard. L'hémolymphe utilisée comme milieu de culture n'est pas responsable du manque de virulence. Bien que le sérum de chaque espèce contienne des principes bactéricides et agglutinants, seule l'agglutinine de crabe des neiges agit contre *A. viridans* (var.) *homari*. Les auteurs examinent la résistance de ces espèces de crabes en relation avec leur potentiel comme réservoirs d'infection.

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Aerococcus viridans (var.) *homari*, formerly *Gaffkya homari* (Editorial Secretary 1971; Kelly and Evans 1974; Buchanan and Gibbons 1974; Stewart and Arie 1974), causes a fatal infection, gaffkemia (Snieszko and Taylor 1947; Hitchner and Snieszko 1947), resulting in periodic heavy mortalities among lobsters (genus *Homarus*). The literature on this disease was reviewed recently by Stewart and Rabin (1970) and Snidermann (1971).

Lobsters became infected in the natural environment (Stewart et al. 1966); however, the source of the infection is unknown. The crab *Cancer irroratus*, which inhabits the same grounds as the lobster and contributes to its diet (Wilder 1966), was shown by Cornick and Stewart (1968b) to be mildly susceptible to the disease, suggesting that it may be a potential reservoir of infection. Other crab species also may be carriers. Accordingly, we examined the pathogenicity of *A. viridans* (var.) *homari* for two other species, the red crab (*Geryon quinquedens*) and the snow crab (*Chionoecetes opilio*), the influence of temperature on pathogenicity, and certain aspects of the hosts' defenses.

Materials and methods — It was necessary to determine the temperature tolerance of the red crabs before they could be used for pathogenicity studies. Groups of 14 crabs, previously acclimated at 2 C for 17 days, were held unfed in tanks containing 250 liters of aerated flowing sea water, maintained within ±0.5 C of the six test temperatures, 12.0, 14.0, 15.0, 16.0, 17.0, and 19.0 C. Mortalities were recorded daily.

Red crabs that had been acclimated for 60 days at 5, 10, and 15 C, and snow crabs similarly acclimated to 5 C were used for the experimental infections. The bacterial suspensions used for infection were prepared from infected lobsters as outlined previously

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TABLE 1. Mortality[a] of red crab (*Geryon quinquedens*) held at various water temperatures.

Time (*days*)	Cumulative no. dead at					
	12 C	14 C	15 C	16 C	17 C	19 C
0	0	0	0	0	0	0
1	0	1	2	1	2	3
2	1	1	2	2	5	4
3	1	1	3	3	5	6
4	1	1	4	4	5	7
6	1	1	4	4	7	7
10	1	1	5	4	7	9
17	1	2	5	4	9	9
30	1	2	6	5	10	9
60	1	2	6	5	10	9

[a]Deaths per 14 crabs (initially acclimated to 2 C) in 250 liters of flowing (1 liter/min) sea water at each test temperature.

(Cornick and Stewart 1968b). Groups of 10 animals at each temperature received doses per crab of 3.0×10^6 bacteria (in 3.0% NaCl solution)/kg body weight, injected into the hemolymph. Control groups received similar volumes of sterile 3.0% NaCl solution. The crabs were held unconfined and unfed in recirculated sea water at the various temperatures. The experiment was terminated after 60 days for the red crab and 100 days for the snow crab. Stained blood smears were examined, the pathogen was reisolated (Stewart et al. 1966), and numbers in the hemolymph were determined (Miles and Misra 1938) at death or at the termination of the experiment.

Methods outlined previously (Cornick and Stewart 1968a) were used to determine the in vitro growth of *A. viridans* (var.) *homari* at 15 C in serum pools (10 animals) from each crab species and the lobster.

Bactericidal activity (Stewart and Zwicker 1972) was determined on serum pools (10 crabs each) from normal and *A. viridans* (var.) *homari* vaccinated (Stewart and Zwicker 1972) red crabs held at the three test temperatures using *Pseudomonas perolens* and *A. viridans* (var.) *homari* as the test microorganisms. Similar tests on normal and *P. perolens* vaccinated (Stewart and Zwicker 1972) snow crabs utilized only *P. perolens* as the test microorganism. Vaccination consisted of injection of 0.5 ml of a formalized suspension of approx. 2.0×10^9 bacteria/ml in 3.0% NaCl. The red crabs were bled 60 days postinjection and the snow crabs after 6 days.

Serum pools from 10 normal red and 10 normal snow crabs held at 5 C were tested for agglutinin activity (Cornick and Stewart 1968a) using standard *Brevibacterium* sp. test microorganism as well as a virulent strain of *A. viridans* (var.) *homari*.

Results — Temperature tolerance data showed that red crabs can withstand water temperatures up to 14 C without excessive mortality. Total mortalities after 60 days at 12, 14, 15, 16, 17, and 19 C were 1, 2, 6, 5, 10, and 9 crabs, respectively (Table 1).

The results of the pathogenicity studies appear in Table 2. Deaths occurred in both crab species, but the numbers of microorganisms in the hemolymph were considered too low to have caused death. Gaffkemia in the lobster, for example, is a bacteremia, and levels of at least 10^9 bacteria/ml hemolymph are normally observed at death. Thus we believe the deaths occurred from causes other than infection with *A. viridans* (var.) *homari*. We were able to reculture the microorganism in broth from a proportion of both crab species at all temperatures. In the case of red crabs, increased water temperature resulted in higher frequency of reisolation and increased numbers of bacteria in the hemolymph as we expected.

When the microorganism was reisolated from both crab species at the termination of the infectivity experiment and inoculated into groups of 10 lobsters, the mean time to death of 12 days was unchanged from its initial value, indicating no loss of virulence while in the crabs.

The in vitro growth of *A. viridans* (var.) *homari* at 15 C in serum pools from both crab species and the lobster *Homarus americanus* is given in Fig. 1. Growth in both crab sera was as good or better than in lobster serum.

We also tested normal red and snow crab sera for the presence of bactericidins (Acton et al. 1969; Stewart and Zwicker 1972) and agglutinins (Cornick and Stewart 1968a; Cornick and Stewart 1973), defense principles active in lobster serum. Serum from both crab species contained activity against the test organism, *P. perolens*, but not

TABLE 2. Pathogenicity and in vivo growth[a] of *Aerococcus viridans* (var.) *homari* in red crabs (*G. quinquedens*) and snow crabs (*Chionoecetes opilio*) at various temperatures.

Crab species	Water temp (C)	No. crabs	No. dead	No. showing positive broth[b]	*A. viridans* (var.) *homari* mean no./ml hemolymph
Red	5	10	2	2	<40
	10	10	0	2	2.5×10^3
	15	10	3	4	3.6×10^5
Snow	5	10	5	10	<40

[a]Treated groups received a dose of 3.0×10^6 bacteria in 3.0% NaCl solution/kg body weight injected into the hemolymph. Control groups received similar volumes of sterile 3.0% NaCl solution. Duration of experiments: red crab – 60 days; snow crab – 100 days. Values include both mortalities and sacrificed crabs. No deaths at each temperature in 10 control red crabs; two deaths with no isolation of *A. viridans* (var.) *homari* in 10 snow crabs. No crabs showed positive hemolymph smears.

[b]Red crab — phenyl ethyl alcohol broth; snow crab — trypticase soy broth.

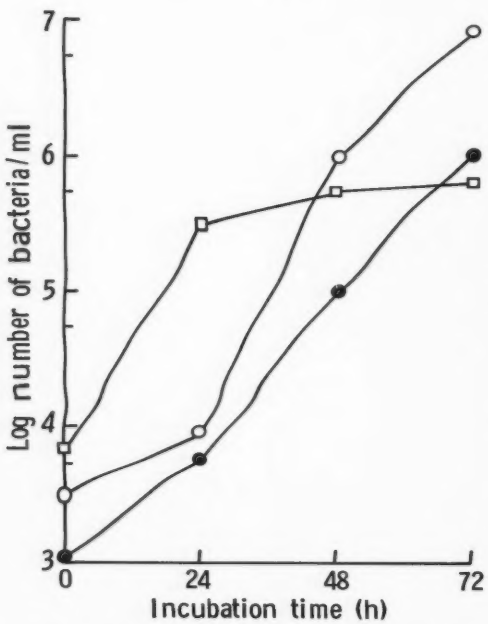

FIG. 1. In vitro growth of *Aerococcus viridans* (var.) *homari* at 15 C in the hemolymph serum of the lobster *Homarus americanus* (●); red crab, *Geryon quinquedens* (□); snow crab, *Chionoecetes opilio* (○).

against *A. viridans* (var.) *homari* (Table 3). Bactericidal titers against *P. perolens* in both vaccinated crab species were slightly increased at 5 C but decreased at 10 and 15 C in the red crab.

Both red and snow crab serum agglutinated the test microorganism (*Brevibacterium*) with titers of 2 and 64, respectively, but only snow crab serum agglutinated *A. viridans* (var.) *homari* (titer 8).

Discussion — The data indicate that the lobster pathogen can remain viable and infective in red and snow crabs for periods up to 60 and 100 days, respectively. This microorganism's lack of pathogenicity for these crab species is not due to the inadequacy of the hemolymph as a growth medium because it grew equally well in vitro in the crab serum as it did in the lobster serum, the species in which it causes a bacteremia. Loss of virulence with extended residence time in the crab was also ruled out as a possible explanation. Although bactericidal activity was present in the hemolymph of both species, it afforded no protection because it was non-*A. viridans* (var.) *homari*-specific. We earlier demonstrated agglutinins against this microorganism in the crab *Cancer irroratus* (Cornick and Stewart 1968b) and suggested that these may play a role in its relative resistance to infection as no such *A. viridans* (var.) *homari*-specific agglutinins are present in the gaffkemia-susceptible lobster. Significantly, the snow crab also possessed similar agglutinins that would lend support to our theory. The resistance of red crabs in the absence of a specific agglutinin is unexplained. However, the important mechanism of phagocytosis (Cornick and Stewart 1968a; Paterson and Stewart 1974), which was not examined in this present work, may offer a possible explanation and is probably operative in all these species.

The host–parasite relationship is a delicate balance resulting in either destruction of the host or the establishment of the more subtle and dangerous carrier state that favors the perpetuation

TABLE 3. Bactericidal activity in red crab (*G. quinquedens*) and snow crab (*C. opilio*) hemolymph serum at various water temperatures.

Species	Water temp (*C*)	Bactericidal titer against *P. perolens* control	*P. perolens* vaccinated	*A. viridans* (var.) *homari* control	*A. viridans* (var.) *homari* vaccinated
Red crab[a]	5	1280	2560	0	0
	10	5120	2560	0	0
	15	5120	1280	0	0
Snow crab[b]	5	640	1280	0	ND

[a]Controls consisted of serum pools from 10 uninjected crabs. Vaccinated consisted of serum pools from 10 crabs 60 days after vaccination with formalin killed *A. viridans* (var.) *homari* in 0.9% NaCl and 0.3% (vol/vol) formaldehyde.

[b]Controls consisted of serum pools from 10 crabs 6 days after injection with 0.3% (vol/vol) formaldehyde in 0.9% NaCl solution. Vaccinated consisted of serum pools from 10 crabs 6 days after receiving a dose of 1.9×10^9 formalin killed *P. perolens* in 1.0 ml of 0.3% (vol/vol) formaldehyde in 0.9% NaCl solution.

ND — Not done.

of the pathogen in nature. We think that the source of the infective agent causing gaffkemia in lobsters is not obvious because of the asymptomatic nature of the infection in the reservoir species, which probably are part of the diet. We have now shown that three crab species, *C. irroratus*, *Chionoecetes opilio* and *G. quinquedens*, can carry the lobster pathogen in a viable state for extended periods of time. We believe that this microorganism is capable of existing in many more marine species. Plainly more research is needed to establish other possible sources of infection.

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Nonselective Feeding of *Pseudocalanus minutus*

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POULET, S. A., AND J. P. CHANUT. 1975. Nonselective feeding of *Pseudocalanus minutus*. J. Fish. Res. Board Can. 32: 706–713.

Feeding by adult females of *Pseudocalanus minutus* on naturally occurring particles was determined by comparing particle size spectra in the water and that removed from it as the ration. Mean diameters and variances computed for the overall particle spectra were similar in both water and ration. Cumulative frequencies of particle numbers and volumes computed for both water and ration were not significantly different in two thirds of the experiments. In one third of the experiments significant values were found, mainly due to addition of particles in the small size ranges, as a result of feeding. The hypothesis of nonselectivity by *Pseudocalanus minutus* was not rejected by the Kolmogorov-Smirnov's test. It is suggested that when particle peaks occur copepods obtain a part of their ration from the most productive cells of the phytoplankton population.

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La nutrition des femelles adultes de *Pseudocalanus minutus* a été étudiée en comparant les spectres de distribution des tailles des particules dans l'eau et dans la ration alimentaire. Les diamètres moyens et les variances calculés pour l'ensemble des spectres se sont avérés semblables dans les deux cas. Dans deux tiers des expériences, les fréquences cumulées du nombre et du volume des particules dans l'eau et la ration n'étaient pas significativement différentes entre elles. Dans un tiers des expériences, des valeurs significatives ont été obtenues, dues principalement à l'addition de particules dans les petites classes de taille par suite de l'action du broutage. L'hypothèse de la non-sélectivité chez *Pseudocalanus minutus* n'est pas rejetée par le test de Kolmogorov-Smirnov. Les résultats suggèrent que les copépodes obtiennent une partie de leur ration alimentaire des cellules les plus productives de la population phytoplanctonique responsable des pics observés dans les spectres de taille.

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RECENT works on food consumption among filter-feeder copepods have been focused on the particle size selection (Mullin 1963; Richman and Rogers 1969; Wilson 1973). Copepods are generally considered to be capable of selecting their food; of consuming preferentially the largest-sized items available in the food mixture offered. However, copepods feeding under natural conditions encounter a particle distribution where concentration and number per unit volume are roughly inversely proportional (Sheldon and Parsons 1967). This

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TABLE 1. *Number of particles ingested related to particle volumes ingested by Pseudocalanus minutus in five size ranges. Theoretical consumption was arbitrarily set at 1.5. Volume and number of particles ingested are in terms of mg × L^{-1} × copepod^{-1} × h^{-1} and L^{-1}, respectively. (Number of experiments in brackets; from Poulet 1974.)*

	Size range (μ)				
	1.58–3.57	4–8.98	10.1–22.6	25.4–57	64–144
	Theoretical consumption				
Particle volume	1.5	1.5	1.5	1.5	1.5
Particle number	19,894	716	86	2	~1
	Average consumption				
Particle volume	0.35	0.89	1.57	4.43	2.03
Particle number	4,761	426	90	6	1
	(60)	(80)	(89)	(84)	(73)

distribution of suspended material is likely responsible for the copepod feeding behavior. Following the methods described by Parsons et al. (1967, 1969) Poulet (1973, 1974) has shown that *Pseudocalanus minutus* adapts its food intake to certain particle concentration within the particle size spectrum. It was also shown that *P. minutus* benefited more from nonselective feeding than from selective feeding. In this report, experimental and computational results are presented which clearly demonstrate that *P. minutus* is a nonselective particle feeder.

Materials and methods — To illustrate and test the relation between the particle distribution in sea water and the feeding response of copepods, several size frequency spectra of particles were selected from the grazing data of Poulet (1974). Three experiments were selected on a seasonal basis and tested to show the influence of the major changes occurring in the spectra with time. Statistical tests were performed on results selected on a monthly basis from experiments reported earlier (Poulet 1974). The size spectra in the control and in the individual ration were compared. The ration per individual was equivalent to the difference between control and experimental water, divided by the number of animals and the experimental time (Poulet 1973).

If *P. minutus* is a nonselective particle feeder, the patterns of particle number and particle volume distribution for each particle diameter should be similar in both control and ration. However, the particle number-frequency and particle volume-frequency distributions are not log-normal, so that nonparametric tests (Median test: Tate and Clelland 1957; Kolmogorov-Smirnov's test: Hollander and Wolfe 1973) were used to examine the hypothesis mentioned above. The cumulative frequencies were computed for each particle size in terms of either particle number or particle volume. Then, differences in the cumulative frequency were evaluated for each particle size between control and ration. The largest difference D_{max} is given by the following equation (Hollander and Wolfe 1973):

$$D_{max} = \mathrm{Max}_{(i)} \mid C_{(i)} - R_{(i)} \mid ,$$

where C is the cumulative frequency in the control, R is the cumulative frequency in the ration, i is the rank (1–20 or 1–40) of a particle defined by its spherical diameter ranging from 1.58 to 144 μ, and where Max is the maximum difference. The median test or χ^2 (Tate and Clelland 1957) is given by the equation:

$$\chi^2 = \frac{N(|BC-AD|-N/2)^2}{(A+B)(C+D)(A+C)(B+D)},$$

where A, C, B, and D are the cumulative frequencies below and above the median of each size spectrum in control (A, B) and ration (C, D), respectively, and where N is the total equivalent to 100%.

Addition of small particles resulting from destruction of larger particles during feeding was often noticed. When this occurred, results showed negative values for the copepod ration within one or several size ranges. The negative "ration" corresponding to each particle diameter where the input occurred was set arbitrarily at zero. One disadvantage of this method was the creation of an artifact in the cumulative frequencies of the ration curve. This input of material artificially increased the difference in the D_{max} values between ration and control.

Results — The average consumption by *P. minutus* is given in Table 1. Theoretically, the same ration arbitrarily set at 1.5 × 10^{-4} mg × copepod^{-1} × h^{-1} could be obtained from any size categories. However, the number of particles ingested would need to be 10^4 times higher in the small range than in the largest one. In nature, because of the variability in the relation between particle number and particle volume, the same volume of food is probably not obtained simul-

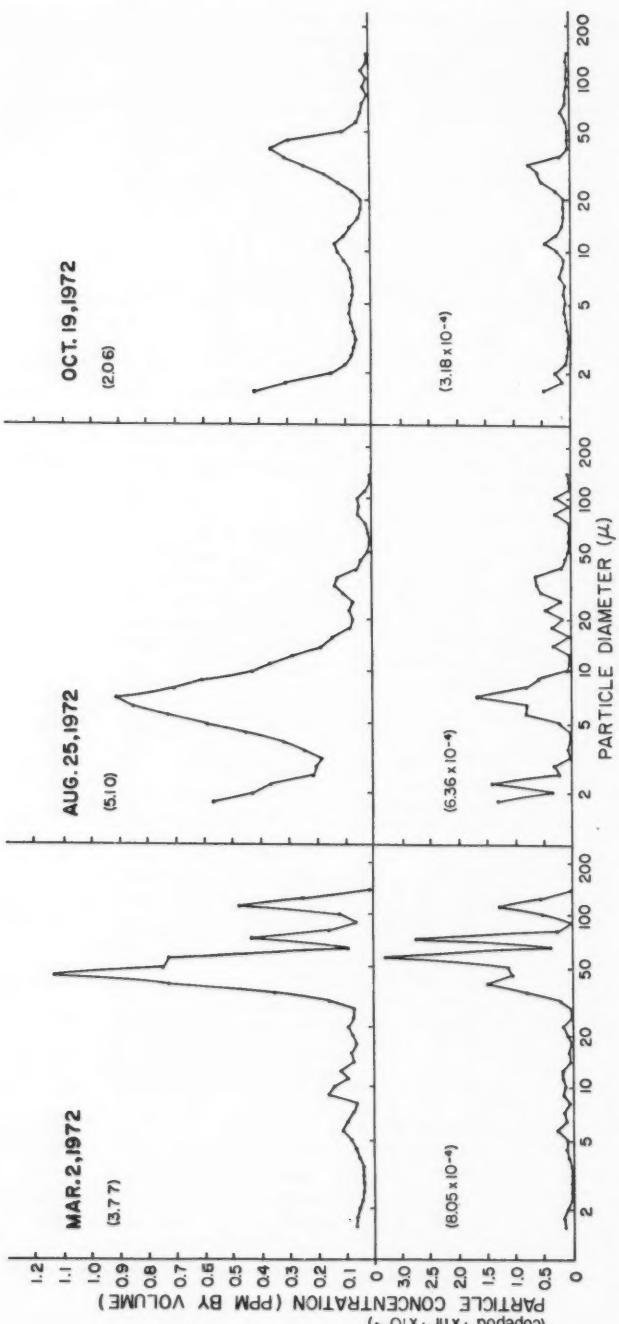

FIG. 1. Size frequency spectra in control and ration. Total particle concentration and food uptake by *Pseudocalanus minutus* are in parentheses. (Feeding experiments from Poulet 1974.)

taneously from all particle size categories. On the average, the consumption expressed in terms of volume increased roughly by a factor of 2, whereas the number of particles ingested decreased by a factor of 10 in each consecutive size range, up to 57 μ (Table 1).

Figure 1 shows some selected experimental results (Poulet 1974) that illustrate three characteristic features of the particle size spectra in sea water (control) and the feeding response of *P. minutus* (ration). The mean particle diameters and their variances have been computed for each size distribution in the three experiments. In each case, the values were similar for both control and ration. When the means and variances were computed in terms of particle number, they were generally less than 5 μ, whereas they fluctuated from one size range to another when expressed in terms of particle volume depending on the particle-volume distribution in the spectra. For copepod feeding, these results mean that the frequency of encountering and consuming a great number of particles is very high in the range below 10 μ. In contrast, the frequency of encountering and consuming a specific volume of food fluctuates with the volume distribution in the particle spectrum.

Figure 2 illustrates the cumulative frequency distributions of particle number (A) and particle volume (B) for both controls and rations. The values of χ^2 and D_{max} between control and ration were not significantly different ($P = 0.05$) when expressed in terms of number, but were significantly different for the March experiment ($P = .016$) when computed in terms of particle volume (Fig. 1). Thus, the Kolmogorov-Smirnov's test (D_{max}) has been performed on data from 42 feeding experiments (Poulet 1974). The tests based on particle volume appeared to be more sensitive than on particle number. The same proportion (~⅓) of significant differences ($P \leqslant 0.05$) has also been found between controls and rations (Table 2). However, in testing these results it was evident that significant D_{max} values were due to addition of particles through feeding mechanisms and breaking up of larger particles. When the addition of particles was low (~5%) or scattered over the entire size spectrum, the values of D_{max} were always nonsignificant. Conversely, in one third of the experiments the input was high (~5–25%) or grouped in a narrow particle size range. Therefore, these tests gave significant values that were always due to the addition of particles generally located in the small size range (<10 μ).

Discussion — It is generally believed that copepods select preferentially the largest-sized items available in the food mixture offered. Figure 3 illustrates two theoretical experiments assuming selective feeding by *Pseudocalanus* on large size particles. Assuming that *Pseudocalanus* is a selective feeder, experimental results should be similar to illustration in Fig. 3 (line 3, left). Assuming that particle concentrations in the control were either unimodal (1) or equal over the entire particle size range (2), then the cumulative frequencies of the particle concentrations between control and ration should be different with highly significant D_{max} values (Fig. 3, right).

Table 2 shows that the values of D_{max} never reached such high values, even in one third of the experiments where D_{max} was always less than 45.5. The size frequency spectra in the ration in any of the experiments were not similar to the patterns shown in Fig. 3. When significant values of D_{max} occurred (Table 2), they were associated with an addition of particles, and the particle spectrum in the ration still had the same patterns as the spectrum in control (Fig. 1, March 2, 1972).

Ivlev suggests in his theories (1945) that animals become more selective as food becomes more available. In laboratory experiments where copepods are fed with phytoplankton cultures, food is usually abundant; hence, selective feeding takes place. When food is scarce there may be no selection. In nature, an abundant stock of particles corresponds generally to uni- or polymodal particle spectra with several concentration peaks, whereas a low concentration generally produces flat particle spectra. In both cases, the diversity of particles in the particle size range is high in terms of size, shape, and "species" composition. When concentration peaks occur, the particle diversity in the spectrum remains almost the same, whereas a few particle categories become dominant. Providing that copepods encounter particles by chance, the intake of food should be random and the intake of a given particle category would be proportional to its concentration in the water. This has been demonstrated in grazing experiments where zooplankton generally consumed the particles causing the peaks (Parsons et al. 1967; Poulet 1973, 1974; Berman and Richman 1974). Conversely, it has been shown that feeding was spread over a larger range within the particle spectrum when concentrations were low (Poulet 1973, 1974). When the experimental results are tested statistically, they do not indicate any selectivity patterns either when food

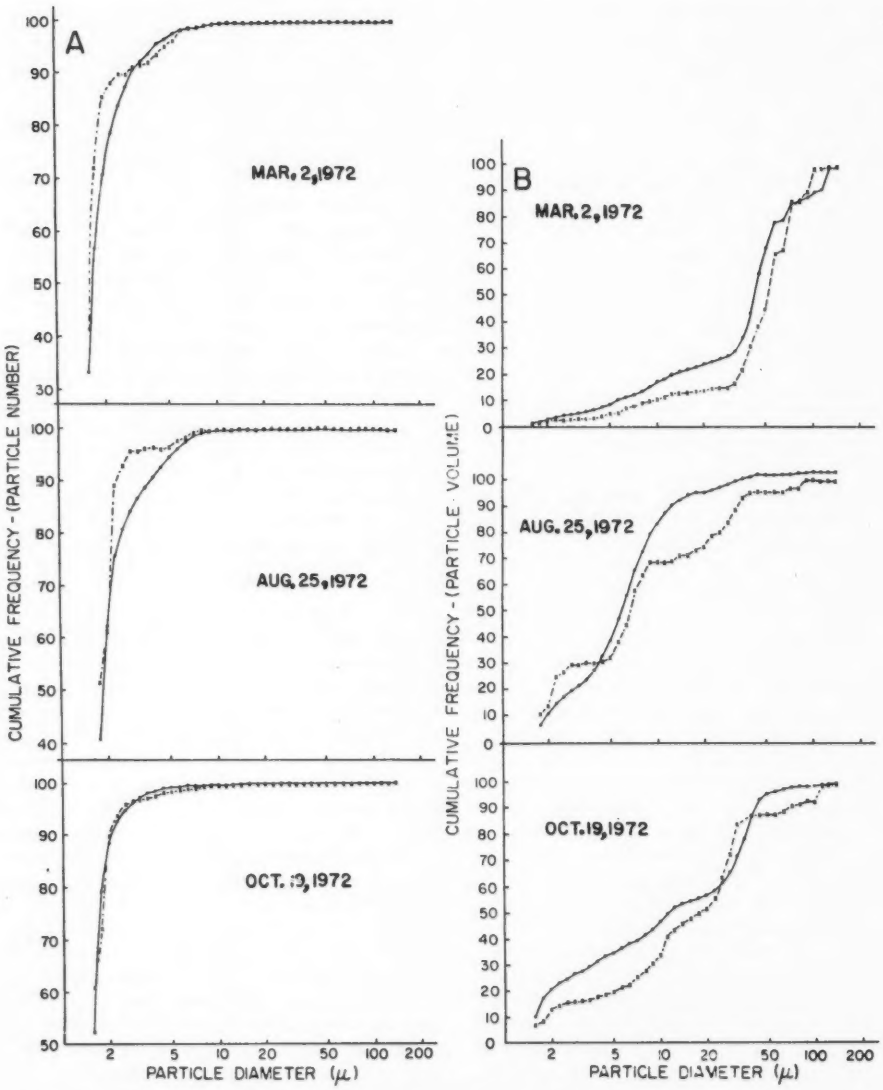

FIG. 2. Comparison between cumulative frequencies in both control (.——.) and ration (x - - - - - x): A, particle number; B, particle volume.

is abundant or scarce; the exception being the 33% of the experiments where particles were actually produced in considerable amounts.

According to Wilson (1973), feeding would seem more advantageous in terms of efficiency when copepods capture larger and larger size particles. In that case, however, copepod feeding would not be particularly well adapted to the particle structure in sea water. A lot of edible particles would be discriminated against by the posture of the feeding appendages. If selectivity really occurs, it should be based on characteristics other than size or "total" particle abundance. As the copepods may not sense food until it comes in

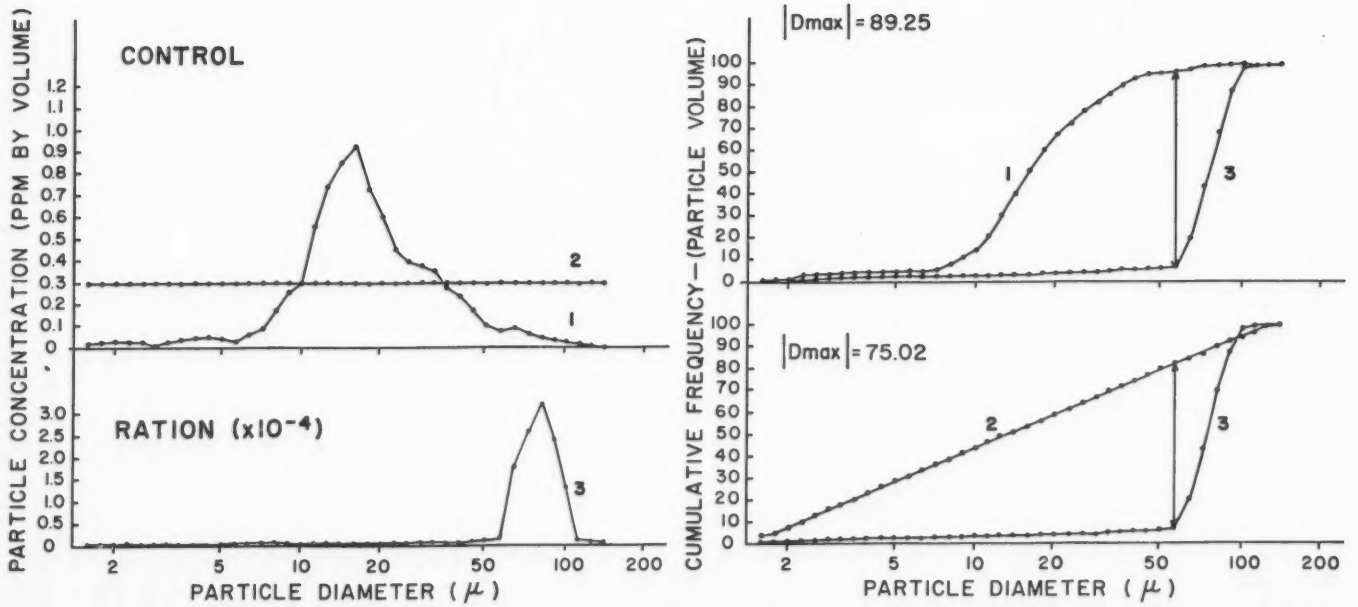

FIG. 3. Theoretical grazing experiments showing selectivity in copepod feeding in the large particle size range: *Left* — size spectra in controls (1 and 2), and in the ration (3); *right* — cumulative frequencies of the particle volume in controls (1 and 2), and in the ration (3). Size spectra in upper left graph are for two different cases, lower graph shows ration obtained by copepod in both cases. The arrows correspond to D_{max}.

TABLE 2. Values of D_{max} computed in terms of particle volume from grazing experiments performed at several seasons. (Particle size range: 1.58–144 μ; number of experiments: 42; from Poulet 1974.) *Significant at 5% confidence level; ** significant at 1% confidence level; N number of particle sizes measured in each experiment.

Date of experiment	D_{max}	N	Date of experiment	D_{max}	N
Mar. 10/71	18.51	20	July 27/72	29.11	40**
Mar. 15/71	18.59	"	Aug. 17/72	45.50	"**
Apr. 1/71	26.32	"	Aug. 25/72	23.23	"**
Apr. 7/71	12.40	40	Aug. 31/72	19.38	"
May 12/71	26.58	"**	Sept. 8/72	32.94	"**
June 1/71	34.36	"**	Sept. 15/72	19.26	"
June 8/71	11.55	"	Oct. 2/72	17.43	"
July 14/71	29.48	"**	Oct. 10/72	20.14	"
Aug. 17/71	12.59	"	Oct. 12/72	12.71	"
Sept. 7/71	21.74	"*	Oct. 16/72	11.90	"
Sept. 14/71	15.19	"	Oct. 19/72	15.24	"
Sept. 29/71	13.77	"	Oct. 30/72	12.31	"
Feb. 28/71	10.89	"	Nov. 6/72	10.94	"
Mar. 2/71	24.48	"*	Nov. 9/72	23.39	"*
Mar. 20/72	15.70	"	Nov. 14/72	20.03	"
Apr. 24/72	26.35	"**	Nov. 20/72	18.95	"
Apr. 29/72	19.96	"	Dec. 7/72	24.63	"*
Apr. 10/72	6.82	"	Dec. 27/72	25.11	"*
Apr. 17/72	13.84	"	Jan. 4/73	18.93	"
May 8/72	31.54	"**	Jan. 11/73	12.53	"
May 18/72	33.27	"**	Jan. 24/73	29.28	"**

contact with the mouth parts (Conover 1966), it is likely that selective feeding, when it occurs, is based on chemical sense.

Phytoplankton is generally responsible for the peaks in particle concentration observed in sea water (Sheldon and Parsons 1967; Poulet 1974). In growing cultures of phytoplankton, the cell size variations are time-dependent (Prakash et al., 1973), but differ from one species to another (Smayda and Boleyn 1966a, b). Nevertheless, this occurs simultaneously with parallel changes in the amplitude and mode of the peak concentration within the size spectrum (Parsons 1965; Prakash et al. 1973). Providing that *P. minutus* is a nonselective particle feeder, then its nutrition should derive mainly from peaks in the spectrum of particle volume, following the dynamics of phytoplankton-cell growth. It is likely then, that it feeds on the most productive cells of the phytoplankton population and probably the most nutritious. This hypothesis has been already documented in *Calanus helgolandicus* which feeds on dividing cells in a synchronously growing diatom culture (Richman and Rogers 1969). Therefore, nonselectivity should allow *P. minutus* to obtain at times a part of its food uptake directly from living cells of potentially nutritive value higher than refractory detritus.

Acknowledgments — We are grateful to Dr R. J. Conover in whose laboratory the work was carried out and whose criticism and advice were greatly appreciated. Thanks are due to Mr M. Paranjape who helped in the writing and discussion and Mrs T. Lecomte and Mr M. Morissette for their assistance in the preparation of the manuscript.

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Effects of Water Temperature and Housing Conditions upon the Aggressive Behavior of the Lobster *Homarus americanus*

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HOFFMAN, R. S., P. J. DUNHAM, AND P. V. KELLY. 1975. Effects of water temperature and housing conditions upon the aggressive behavior of the lobster *Homarus americanus*. J. Fish. Res. Board Can. 32: 713–717.

Lobsters are more aggressive at 10 C than at 5 C. At 10 C, animals housed communally, and those in physical isolation with visual contact, are less aggressive in a test situation than lobsters housed with no physical or visual contact.

HOFFMAN, R. S., P. J. DUNHAM, AND P. V. KELLY. 1975. Effects of water temperature and housing conditions upon the aggressive behavior of the lobster *Homarus americanus*. J. Fish. Res. Board Can. 32: 713–717.

Les homards sont plus agressifs à 10 C qu'à 5 C. A 10 C, les homards logés en communauté et ceux physiquement isolés, mais avec contacts visuels, sont moins agressifs, dans une situation expérimentale, que les homards logés sans contacts physiques ou visuels.

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THE American lobster (*Homarus americanus*) is known to be a solitary, aggressive animal in its natural environment (Herrick 1896, 1911). When lobsters are crowded together in high density holding units such as lobster pounds, mutilations received during aggressive interactions and concomitant cannibalism constitute one of the major problems for commercial holding or aquaculture operations (McLeese 1972). Despite this problem, there have been few published reports of specific efforts to reduce this intraspecific aggression, or to describe the variables which affect the amount of such aggression.

Dunham (1972) reported that lobsters held in a communal housing tank for 36 days showed less aggression, when subsequently tested against a target lobster, than lobsters housed in individual compartments. The particular aspect of the communal housing condition responsible for the reduction in aggressive behavior was not determined.

The present experiment was designed to determine if physical contact between lobsters was necessary to reduce aggression or if simple visual

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exposure to conspecifics (without physical contact) was sufficient. The aggressive behavior of lobsters housed individually with physical and visual isolation from other lobsters (Group IH) was compared with lobsters housed individually in physical isolation but with visual contact (Group VH), and with lobsters housed communally (Group CH).

In addition to housing conditions, other environmental factors might be expected to affect the aggressive behavior of the lobster. As water temperature has been shown to influence other aspects of the lobster's behavior (McLeese and Wilder 1958), two temperatures (5 and 10 C) were combined factorially with the housing conditions to assess the effects of both factors upon the aggressive behavior.

Materials and methods — Twenty-five male lobsters (averaging 100 mm ± 5 mm carapace length) were obtained from the Fisherman's Market, Halifax, N.S. They were judged to be in the hard shell stage C of the intermolt cycle (Passano 1960). Rubber bands immobilizing the chelae were removed when the animals were placed in the laboratory holding tanks. One lobster was selected arbitrarily as the target animal and placed in the testing tank. The other lobsters were randomly assigned to three groups and placed in tanks with the appropriate housing conditions (IH, VH, and CH).

The eight lobsters in Group IH were placed in individual compartments 16 cm wide, 30 cm long, and 30 cm deep, formed by placing perforated plywood dividers in a long tank. Animals in Group VH were housed in identical compartments except that the dividers were transparent plexiglass. Animals in Group CH were housed together in a single large tank 100 cm wide, 80 cm long, and 55 cm deep.

All tanks were constructed of marine plywood with a light gray epoxy paint finish. A plateglass front in each tank permitted observations of the lobsters. The tanks were supplied with flowing unfiltered sea water originating from the Northwest Arm of Halifax Harbor. Flow rate was approximately 1.9 liters/min in the communal tank and 3.6 liters/min in the other tanks. Temperature of the sea water remained between 4 and 6 C through the first test series, and gradually increased to 10 C before the second test series was run. A 12-h day–night cycle was maintained with the lights coming on at 0730 daily. The animals were fed live mussels (*Mytilus edulis, Modiolus modiolus*), and sea urchins (*Strongylocentrotus drobachiensis*).

Animals were left in the differential housing conditions for 36 days before the first test series was conducted.

Test procedure — The test procedure was that used previously by Dunham (1972). Briefly, the test tank was 100 cm wide, 80 cm long, and 55 cm deep, with a plateglass front. A single shelter was formed with two flat rocks against the standpipe in a rear corner. The test procedure consisted of two parts. First, the target lobster was temporarily removed from the test tank and each of the 24 test lobsters was placed alone in the test tank for 30 min. The amount of time each lobster spent exploring the tank before settling in the shelter was recorded. Eight lobsters were tested from 1730 to 2130 each day during days 37–39 of the experiment. Order of testing was counterbalanced across the three groups. The test tank was illuminated with a 100-W incandescent bulb suspended 90 cm over the center of the tank.

Secondly, after the activity measurements had been made, the target lobster was replaced in the test tank. Then, on days 40–42 each test lobster was again placed in the test tank for a 30-min period, during which the frequency and duration of all attacks it initiated against the target lobster were recorded. Onset of an aggressive attack was defined as that point when physical contact was made between the two animals and termination of the attack was when contact was broken (Dunham 1972).

The lobsters were maintained in the appropriate housing conditions until the temperature of the sea water stabilized at 10 C. Then, on days 129–131 the second set of activity tests were conducted, and on days 132–134 the aggression tests were again conducted.

After the first activity test series (5 C), one lobster was eliminated from the CH group due to physical damage incurred in the communal tank. It was replaced to maintain the population density, but data from the new lobster were not collected. A breakdown in the seawater system resulted in the death of two animals from the IH group between the first and second test series. To simplify the analysis, data for the missing cells in the analysis of variance were generated using a least-squares method (Snedecor and Cochran 1967) and appropriate alterations were made in degrees of freedom.

Results — Activity test — Although the time spent exploring the test tank varied widely among the 24 lobsters, only two animals failed to settle in the shelter by the end of the 30 min. Animals at 10 C tended to explore slightly longer than at 5 C (910.4 s vs. 683 s) and those in Group CH longer than the other groups, but there were no significant differences in exploration time across either temperatures ($F = 1.84$) or housing conditions ($F = 1.27$).

Aggression test — Aggressive contacts occurred more frequently at 10 C than at 5 C ($F = 15.39$, $P < .01$) (Fig. 1). Furthermore, more time was spent in aggressive interactions at 10 C than at 5 C ($F = 13.07$, $P < .01$) (Fig. 2).

Analyses of variance also indicated that there was a significant difference in the frequency

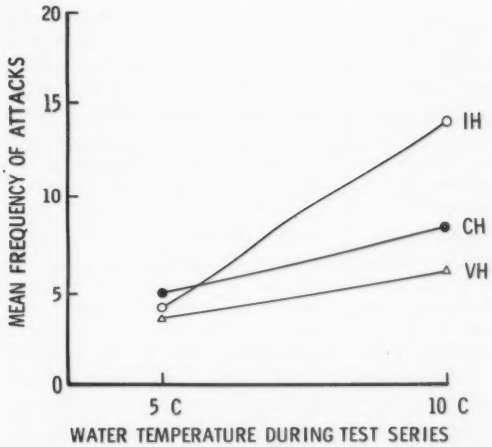

FIG. 1. Mean number of attacks initiated against the target animal by the eight lobsters from the individually housed (IH), communally housed (CH), and visual housing (VH) groups during the 30-min aggression tests.

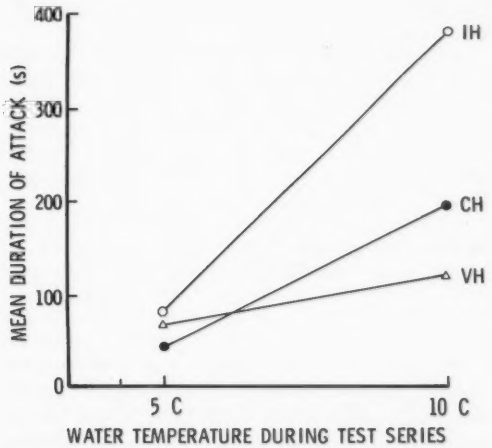

FIG. 2. Mean duration of fighting initiated by lobsters from each of the three housing groups during the 30-min aggression tests. Abbreviations as in Fig. 1.

($F = 3.61$, $P < .05$) and duration ($F = 3.25$, $P < .05$) of aggression between different housing conditions.

Comparisons of individual group means using linear combinations suggest that the effects of both temperature and housing condition are largely accounted for by the high scores for the IH group at 10 C. Both Group CH and Group VH were significantly less aggressive than Group IH on both the frequency ($t = 2.8$, $P < .01$) and duration ($t = 2.98$, $P < .01$) measures of fighting at 10 C. No significant differences were found between various housing conditions at 5 C.

Discussion — It appears from both Fig. 1 and 2 that the housing conditions had an effect only at 10 C. This is not surprising as there is so little aggression at 5 C under any housing condition.[1] The differences at 10 C between Group IH and Group CH essentially replicate the earlier findings of Dunham (1972) that lobsters housed communally (in temperatures ranging from 15 to 17 C) were less aggressive than individually housed animals.

There are, of course, two possible interpretations of these housing effects. First, as suggested, communal housing (or "visual" housing) can be assumed to be the active variable that reduces the "normal" high level of aggression found in lobsters. Alternatively, social isolation may produce an increase in aggression in Group IH relative to Group CH and VH. Although this may seem a fine distinction, there are several known cases among vertebrates where isolation of individuals results in dramatic increases in intraspecific aggression (Valzelli 1973). It is doubtful that a similar mechanism operates among lobsters. Personal observations by SCUBA and data reported by Cobb (1971) support the traditional descriptions that lobsters are solitary-dwelling animals (Herrick 1911). In terms of social history, it may be that the condition of lobsters in our individually housed (IH) group is simply a closer approximation of the lobster's natural condition. The important point is that communal or visual housing conditions produce less aggressive lobsters.

Further experiments completed recently lend support to the assumption that exposure to communal housing is an active variable that reduces aggression. Ten adult male lobsters were housed in isolation for 14 days and then placed together in a communal tank (at 15 C). The frequency of aggressive encounters per animal was observed during the first 3 days of communal housing.

[1]A second group of animals has since been run in the 5 C conditions of the present experiment. The mean number of bouts were 4.1 (IH animals), 1.9 (VH animals), and 3.4 (CH lobsters) during the 30-min test; mean durations of fighting were 78.9 s (IH), 65.1 s (VH), and 86.9 s (CH). Most of these scores are close to those obtained in the present experiment.

Observations were made during the first ½ h of the 12-h daylight period. The mean number of aggressive encounters in the communal tank was 15 on the first day, 13.2 on the second day, and 0.7 on the third day. The extent to which the initially high level of aggression represents the lobster's "natural" aggressive tendencies, or has been induced by isolation in the laboratory, remains to be determined, but there can be no doubt that exposure to conspecifics does lead to a reduction in that aggression. This reduction may result from establishment of dominance hierarchies within the communal tank that would lead to decreased aggressiveness in subordinate animals. Douglis (1946) found that such hierarchies were established in groups of two, three, or four lobsters and that subordinate animals were less aggressive in the presence of a dominant lobster. Larger hierarchies have been reported by Lowe (1956) using the freshwater crawfish *Cambarellus shufeldtii* in groups of up to eight animals.

However, the development of a dominance hierarchy would not explain why the communally housed animals display less aggression in a separate test situation against a "new" or target lobster. That a similar reduction in aggression can be achieved without actual physical contact (Group VH) suggests that the attenuation of aggression produced by the communal housing may be due to a change in the lobster's reaction to visual signals from conspecifics. Certainly other sensory modalities may also be involved; for example, McLeese (1973) has shown that lobsters will react to water from tanks holding other lobsters. Such chemical factors probably had little to do with the present experiment as all three groups had chemical "exposure" to conspecifics. However, a possible role of visual signals is somewhat surprising because the lobster's typical agonistic interaction is not characterized by a rigid sequence of stereotyped visual display (Scrivener 1971), such as that seen in many other decapod crustaceans, particularly certain species of crabs (Hazlett 1972; Wright 1968). The most obvious visual display produced by the lobster preceding an aggressive encounter is a raising and spreading of the chelipeds (meral spread), and even this is not observed consistently. Further research will be necessary to determine whether some specific aspect of the lobster's behavior produces the reduction observed in the aggressive behavior of these animals.

The increased aggressiveness observed at the higher temperature (Group IH) might be attributed to a number of factors. McLeese and Wilder (1958) reported that the lobster's rate of movement increases as water temperature increases. Possibly, the increased aggression observed at the higher temperature is partially a result of increased locomotor activity bringing the animals into physical contact more frequently. However, our measure of activity showed no significant change with temperature in the amount of time lobsters spent exploring the test tank, so any effects due to differential activity should be minimal. Furthermore, not only did the overall frequency and duration of aggressive behavior increase at the higher temperature, but the average duration of a single attack was also higher at 10 C than at 5 C. An increase in locomotor rate at the higher temperature would not predict that, once initiated, contacts would last longer in more active animals.

It seems more likely that the increased aggression under higher temperature conditions is mediated by one of several other mechanisms. It has been our experience, for example, that frequency of feeding increases systematically with increases in water temperature. Considering the lobster's cannibalistic tendencies, increased aggression may simply be related to an increase in food-getting behavior. Certainly, data concerning the effects of food deprivation on aggression would be desirable for this species.

Similarly, seasonal increases in temperature are known to be associated with increases in molt frequency (Hughes and Matthiessen 1967; Hughes et al. 1972). It would be adaptive for the lobster to become more territorial and defensive upon entering proecdysis as the animal is vulnerable to predators during and after the molt.

Although the present data demonstrate that IH lobsters in particular are more aggressive at higher temperatures, the exact mechanism (or mechanisms) responsible for this relationship cannot yet be specified. However, this effect of temperature does suggest, from a practical point of view, that operators holding large numbers of recently captured (and presumably highly aggressive) lobsters for short periods of time (i.e. where molting is not desirable) should supply the holding tanks with sea water of low temperature. Intake pipes, for example, could be located below the thermocline in areas where this is possible. The lower temperature of water from that region may substantially reduce aggressive and cannibalistic behavior and consequent financial losses incurred in commercial operations during the warmwater period in summer and fall.

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The Shell Makers, Introducing Mollusks. *G. Allen Solem. John Wiley and Sons, Inc., New York, N.Y. 1974. xii, 289 p., 94 text figures and 12 colored plates. $9.95.*

"Many popular books have been published on *shells* but very few on *mollusks*." This is the opening statement of the Preface to *The Shell Makers* and it sets the stage for what follows. This is not a book for anyone who wants to identify shells but is rather for those who are interested in mollusks as living organisms. It delves into the various ways these soft bodied animals are adapted to their environment, how they live, feed and reproduce, as well as the various paths of their evolution. To do this in 289 pages is an impossible task but Dr Solem has attempted it. He endeavored to make it a popular account, as chapter headings such as "The Slow and the Quick," "The Filtrationists," "The Road to Land," and "On Becoming Sluggish" would suggest. However, he inevitably ended up using technical terms and scientific names which sent the interested undergraduate biology students on whom I tested the book running for texts on molluscan anatomy and classification. This is not meant to be critical but to point out the difficulty of the task Dr Solem set for himself. There is much that amateurs and nonzoologists can gain from reading *The Shell Makers* even if they have no knowledge of the many genera and species cited as examples, the difference between orthurethrous, mesurethrous, and sigmurethrous kidneys, or the varied patterns of molluscan mantle cavities.

Discussions on the evolution and functional morphology of mollusks are illustrated with interesting vignettes on subjects such as the development of the siphon in predatory neogastropods and its importance in chemoreception, variation among filter feeders and their restriction to the aquatic environment, the plight of freshwater mollusks as a result of damming rivers, and the importance of a humid environment in the evolution of slugs. Such accounts make this a useful book for adjunct reading in a zoology or ecology course.

Dr Solem's theories on the evolution of the Mollusca, spiral coiling and torsion will certainly be questioned by some malacologists. He stated that he was taking a "slightly different approach" and that his ideas were not in accordance with those of Stasek (1972). Unfortunately this subject is far too complicated to discuss in a review but it is suggested that interested readers should also consult Stasek and the many references he cited.

Since Dr Solem is a land snail specialist it is not surprising that his treatment of the smaller classes of mollusks is weak because they are all marine. Even the bivalves and marine gastropods do not receive the enthusiastic, exciting treatment given the land snails, though the majority of the fine colored plates of living mollusks are devoted largely to marine forms. In fact this reviewer would appreciate a book of this type devoted entirely to land snails. The chapters "Experiments in Living" and "To Scrape for a Living" are illustrated with numerous fine-scanning electron microscope pictures of land snail shells and radulae. The chapter on zoogeography is concerned entirely with land snails. Though nearly half of the book is devoted to land snails, much of it based on Dr Solem's original work, one feels the author still has much more he could say about them.

The most serious criticism of the book is the lack of documentation. The selected references on mollusks given in Appendix B are helpful but it would be extremely difficult for the reader to locate a specific topic he wished to pursue. Many of the authors who are vaguely referred to in the text such as A. Bidder, A. Brown, S. Calquist, W. Garstang, M. Ghiselin, S. Kawaguti, A. Lang, A. Naef, B. Peyer, T. Thompson, A. Underwood, A. Wegener, E. Wilson, R. MacArthur, E. Yochelson, and E. Zimmerman are not listed in the references and often not even a date is given to aid in the search. Though I realize this fault is common to many popular books, it is a serious one for it leads to vague, inaccurate quoting. Sometimes when a statement made as fact is pursued to its ultimate source, which may take many hours or days, one finds it was overstated,

wrongly quoted, or the original statement was not based on actual fact. This is not to say that Dr Solem was inaccurate but only that it would take a long time to check his statements or get further data if one was interested.

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Sea Fisheries Research. *Edited by F. R. Hardin Jones. Paul Elik (Scientific Books) Ltd., London. 1974. 510 p. $000.*

This is a large book, 510 pages in all, now dedicated to the memory of Michael Graham. To quote from the preface —

"Graham was a persistent and critical questioner and the suggestion: 'Why not write it down for him?' came as a protective cry from one member of the staff following a particularly searching hour. This book grew from that idea. A small group — Dr. H. A. Cole, Mr. A. J. Lee, Dr. D. H. Cushing, Dr. F. R. Harden Jones and Mr. D. J. Garrod — was responsible for selecting contributors, and Harden Jones dealt with many of the editorial problems. It was hoped to be able to present the book to Michael Graham on his 70th birthday but that was not possible."

The prologue (by the late R. S. Wimpenny) and the epilogue (by R. J. H. Beverton), which are principally concerned with Graham and his impact on the development of fishery research in England, were of particular interest to me. Michael Graham was a special person. The contributors think so too and a Michael Graham Prize is to be established, funded by the royalties received from the sale of this book.

In scope it is eclectic, containing 21 chapters, a prologue, and an epilogue, each prepared by a different contributor. The subjects range from radioactivity in the sea to research vessel design, and from studies of the otolith to the problems of fishery management.

The quality varies widely from high as in the case of Arthur Lee's "Oceanic Circulation of the North Atlantic Region" to less than adequate (for such a volume) as in the case of "World Resources of Hakes of the Genus *Merluccius*" by B. W. Jones. There are some thought-provoking chapters on rather sophisticated aspects of fishery biology including those by Cushing (The Natural Regulation of Fish Populations), Iles (The Tactics and Strategy of Growth in Fishes), Holden (. . . Rational Exploitation of Elasmobranch Populations . . .), and Harden Jones (. . . Research into Fish Behavior). There are also some very useful, if pragmatic, chapters including those by Burd (The North-East Atlantic Herring and the Failure of an Industry), Blacker (Recent Advances in Otolith Studies), and Trout (Development in Research Vessel Design).

The eclectic nature of the volume is in itself a tribute to Michael Graham. It is inevitable, however, that each reader will see it differently, depending upon his background and interests. It is the type of book each of us involved in marine fisheries research will wish to have but not the kind of book that I would recommend a student buy.

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Books Received
December 1974 – February 1975

Chemoreception in Marine Organisms. *Edited by P. T. Grant and A. M. Mackie. Academic Press, London, New York, 1974. xi + 295 p. £7.40 (= about $17.80 Canadian).*
(Multi-authored volume on structure and function of chemoreceptor cells, chemical senses of fishes, chemoreception in marine invertebrates, and factors influencing settling of marine larvae.)

The Early Life History of Fish. *Edited by J. H. S. Blaxter. Springer-Verlag, New York, Heidelberg, Berlin, 1974. xii + 765 p. $40.20.*
(Proceedings of an international symposium at Oban, Scotland, May 1973; includes 63 papers.)

FAO Investigates Ferro-cement Fishing Craft. *Edited by John Fyson. Fishing News (Books) Ltd., West Byfleet, England, 1973. 200 p. £13.50 (= about $32.40 Canadian).*
(Includes 26 papers contributed to an inter-

national symposium in New Zealand; covers material properties, scantlings and regulations, construction methods, and cost and service experience.)

Fish Farming International, No. 3. *Edited by Peter Hjul. Fishing News (Books) Ltd., West Byfleet, England, 1974. 152 p. £3.00 (= about $7.20 Canadian).*
(Third issue of new journal; regular quarterly publication began March 1975.)

The Inland Waters of Tropical Africa. An introduction to tropical limnology. *L. C. Beadle. Lowe & Brydone (Printers) Ltd., Thetford, Norfolk; distributed by Longman Inc., New York, 1974. viii + 365 p. $19.00.*
(Geological and climatic history of the Great Lakes of Africa, distribution and evolution of aquatic faunas, adaptations of organisms to life in tropical waters, circulation of matter and energy, and productivity of tropical lakes.)

La Pêche au Maroc. *Roger Chaban, Éditions Alpha G.E.A.M., 1973. 195 p., illus.*
(Popular treatment of angling techniques and sport fish available in Morocco's major lakes.)

Fiziologo-biokhemicheskie osobennosti godovykh tsiklov ryb. *(Physiological and biochemical characteristics of the annual cycle among fishes.) G. E. Shulman. Pishchevaya Promyshlennost Press, Moscow. 366 p. 2.20 rubles.*
(Reviews on seasonal changes in fat, protein, and ash, and their relationship to age, growth rate, maturation, spawning, and wintering, for 13 species of commercial fishes mainly from the Black Sea region.

The translated title and synopsis of the book were kindly provided by Dr. W. E. Ricker.)

Pollution Ecology of Freshwater Invertebrates. *Edited by C. W. Hart Jr., and Samuel L. H. Fuller. Academic Press, Inc., New York and London, 1974. xiv + 389 p.*
(Multi-authored volume on normal and pollution ecology of major invertebrate groups.)

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Protein instability in minced flesh from fillets and frames of several commercial Atlantic fishes during storage at −5 C — **J. R. Dingle and J. A. Hines**

Utilization of blue whiting, *Micromesistius poutassou*, for human consumption — **B. Dagbjartsson**

Swimming performance of juvenile coho salmon (*Oncorhynchus kisutch*) exposed to bleached kraft pulpmill effluent — **T. E. Howard**

Effects of pH on toxicity of antimycin on fish — **L. L. Marking**

Effects of acute exposure to bleached kraft pulpmill effluent on carbohydrate metabolism of juvenile coho salmon (*Oncorhynchus kisutch*) during rest and exercise — **D. J. McLeay and D. J. Brown**

Effect of DDT on discriminating ability of rainbow trout (*Salmo gairdneri*) — **P. G. McNicholl and W. C. Mckay**

Density of the commercial spider crab, *Chionoecetes opilio*, and calibration of effective area fished per trap using bottom photography — **R. J. Miller**

Quantitative and qualitative studies of bacteria isolated from sea water used in the laboratory culture of the American oyster, *Crassostrea virginica* — **R. A. Murchelano, C. Brown, and J. Bishop**

Mercury in seals, terrestrial carnivores, and principal food items of the Inuit, from Holman, N.W.T. — **T. G. Smith and F. A. J. Armstrong**

Chemical attraction of some marine fish in their natural habitat — **A. M. Sutterlin**

Notes

Selenium toxicity on the early life stages of zebrafish (*Brachydanio rerio*) — **A. J. Niimi and Q. N. LaHam**

Tocopherol composition of frozen Atlantic herring (*Clupea harengus harengus*) tissues and oil — **F. R. Englehardt, J. R. Geraci, and B. L. Walker**

Effects of saline from North Dakota lakes on survival of fathead minnow (*Pimephales promelas*) embryos and sac fry — **B. L. Burnham and J. J. Peterka**

Simplified spectrophotometric curcumin method for the determination of boron in marine shellfish — **R. E. Drew**

Effects of kokanee salmon (*Oncorhynchus nerka*) decomposition on the ecology of a subalpine stream — **J. E. Richey, M. A. Perkins, and C. R. Goldman.**

Perspectives

Reproductive guilds of fishes: a proposal and definition — **E. K. Balon**

Book Reviews

Netting materials for fishing gear (G. Klust) — **P. J. P. Carrothers**

Modern inshore fishing gear — rigging and mending (J. Garner) — **P. J. P. Carrothers**

The poisons around us (H. A. Schroeder) — **J. B. Sprague**

RECENT PUBLICATIONS / RÉCENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed at the end of the section.

Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section.

Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

2032. BUERKLE, U. 1974. Gill-net catches of cod (*Gadus morhua* L.) in relation to trawling noise. Mar. Behav. Physiol. 2: 277–281. (6)

2033. SAUNDERS, R. L., AND E. B. HENDERSON. 1974. Atlantic herring as a dietary component for culture of Atlantic salmon. Aquaculture 3: 369–385. (6)

2034. GARDELLA, E. S., AND A. B. STASKO. 1974. A linear-array hydrophone for determining swimming depth of fish fitted with ultrasonic transmitters. Trans. Am. Fish. Soc. 103: 635–637. (6)

2035. SYMONS, P. E. K. 1974. Territorial behavior of juvenile Atlantic salmon reduces predation by brook trout. Can. J. Zool. 52: 677–679. (6)

Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études, comprenant deux parties. Les études de 1969, partie 2, incluant le n° 1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

2036. SOCHASKY, J. B., AND D. E. AIKEN. 1974. The Y organ — molting gland problem in decapod Crustacea: the "organ of Madhyastha" and "organ of Carlisle," two putative Y organs. Can. J. Zool. 52: 1251–1257. (6)

2037. DINGLE, J. R., AND J. A. HINES. Some enzymic reactions of adenine derivatives in the tail muscle of the lobster, *Homarus americanus*. Comp. Biochem. Physiol. 48B: 1–10. (8)

2038. ZITKO, V., O. HUTZINGER, AND P. M. K. CHOI. 1974. Determination of pentachlorophenol and chlorobiphenylols in biological samples. Bull. Environ. Contam. Toxicol. 12: 649–653. (6)

Interpretive Articles Series

This new series commenced in 1966. The articles interpret work already published in primary journals. They appear at irregular intervals in various technical publications. Frequently an article essentially duplicates, condenses, or is based on an article in another series.

323. JANGAARD, P. M. 1974. Lobsters. By A. H. Johnson (Consultant), M. S. Peterson [ed.], Encycl. Food Technol. 2: 554–557. (12)

Articles explicatifs

Cette série a débuté en 1966. Les articles donnent une explication de travaux déjà publiés dans des revues de fonds. Ils paraissent à intervalles irréguliers, dans diverses publications techniques. Souvent, un article se basera sur un écrit d'une autre série, le condensera ou le reproduira presque tel quel.

Manuscript Reports

This series includes unpublished preliminary reports and data records not intended for general distribution. They should not be referred to in publications without clearance from the issuing establishment and without clear indication of their manuscript status.

CARSON, W. V. 1974. Chemical conditions in the Northwest Miramichi River during 1973. Fish. Res. Board. Can. MS Rep. 1323: 26 p. (6)

ZITKO, V. 1974. Effects of pollutants on marine life. A review of research in Canada in 1973–1974. Fish. Res. Board Can. MS Rep. 1324: 17 p. (6)

Rapports manuscrits

La série comprend des articles d'information et des rapports préliminaires non publiés, qui ne sont pas destinés à la distribution générale. Ils ne doivent pas être donnés en références sans l'assentiment du service dépositaire et sans une indication claire de leur forme manuscrite.

ARO, K. V., AND J. MCDONALD. 1974. Commercial and sport catches of chinook and coho salmon along the west coasts of Canada and the United States (data record). Fish. Res. Board Can. MS Rep. 1325: 91 p. (1)

Technical Report Series

Technical Reports are research documents that are of sufficient importance to be preserved, but which for some reason are not appropriate for primary scientific publication. Inquiries concerning any particular Report should be directed to the issuing establishment.

488. CLARK, R. M. 1974. The effects of effluents from metal mines on aquatic ecosystems in Canada. A literature review. Fish. Mar. Serv. Res. Dev. Tech. Rep. 488: 150 p. (4)

489. THOMPSON, B. 1974. The effects of effluent from the Canadian textile industry on aquatic organisms. A literature review. Fish. Mar. Serv. Res. Dev. Tech. Rep. 489: 99 p. (4)

490. KOHLER, A. C., D. J. FABER, AND N. J. MCFARLANE. 1974. Eggs, larvae and juveniles of fishes from plankton collections in the Gulf of St. Lawrence during 1968. Fish. Mar. Serv. Res. Dev. Tech. Rep. 490: 105 p. (6)

491. ZITKO, V., AND E. ARSENAULT. 1974. Chlorinated paraffins: properties, uses, and pollution potential. Fish. Mar. Serv. Res. Dev. Tech. Rep. 491: 38 p. (6)

Rapports techniques

Les rapports techniques sont des documents de recherche qui revêtent une assez grande importance pour être conservés mais qui, pour une raison ou pour une autre, ne conviennent pas à une publication scientifique de fonds. Pour toute demande de renseignements concernant un rapport particulier, il faut s'adresser au service responsable.

Translation Series

The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. Copies are available from Translation Services, Canada Institute for Scientific and Technical Information, National Research Council of Canada, Ottawa, Ontario K1A 0S2, at a cost of 10c per page and a minimum charge of $1.00. Information on translations can generally be obtained from the Office of the Editor.

PRAVDIA, I. F. 1966. Handbook for the study of fishes (mainly freshwater). p. 98–109; 219–220. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3309, 1974)

POKORNY, J., P. TAI, N. LUAN, AND G. JANICEK. 1973. Reactions of oxidized lipids with proteins. 8. Reactions of alcanals with proteins. Die Nahrung 17: 621–627. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3310, 1974)

JACOB, J., AND G. FRIMMER. 1973. Occurrence of alkylhydroxymalonic acids in uropygial gland secretions of birds. Hoppe-Seyler's Z. Physiol. Chem. 354: 1648–1650. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3311, 1974)

SEHER, A. 1972. Fats in nutrition. Ernaehr.-Umsch. 19: 242–247. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3312, 1974)

NOMURA, M., K. SAKAI, D. INABA, T. NOSE, AND Y. SHIMMA. 1973. Application of imported Peru fish meal in fish feed. IV. Feeding experiment by pellets. Tansuiku Suisan Kenkyusho Kenkyu Hokoku 23: 105–112. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3313, 1974)

TAKAMA, K. 1974. Insolubilization of rainbow trout actomyosin during storage at –20 C. I. Properties of insolubilized protein formed by reaction of propanal or caproic acid with actomyosin. Nippon Suisan Gakkaishi 40: 585–588. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3314, 1974)

ZUEV, G. V., AND K. N. NESIS. 1971. The role of squids in the food chains in the ocean. Chapt. 4: 78–83. *In* Squids — biology and fishery. Izdatel'stvo Pishchevaya Promyshlennost' Moskva (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3315, 1974)

ZORAWSKI, A. 1973. Simplified method of fat sample preparation for the determination of fatty acid content by gas–liquid chromatography. Tluszcze Jadalne 17: 182–185. (Transl. from Polish by Fish. Mar. Serv. Transl. Ser. No. 3316, 1974)

KOZLOWSKA, J., AND A. FIEBIG. 1972. Effect of low storage temperature on stability of medicinal cod-liver oil. Rozpr. Wydz. 3: Nauk Mat. Przyr. Gdansk. Tow. Nauk. 3: 225–229. (Transl. from Polish by Fish. Mar. Serv. Transl. Ser. No. 3317, 1974)

IMAMURA, M. 1973. Treatment of P.C.B. Kagaku (Kyoto) 28: 581–586. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3318, 1974)

DOI, T., K. Y. MUN, K. TAKAO, K. ISHIOKA, AND K. OKADA. 1972. Stock assessment of the Kuruma-Prawn *Penaeus japonicus* Bate in the Hiuchi-Nada trawling grounds. Tokaiku Suisan Kenkyusho Kenkyu Hokoku (69): 45–54. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3319, 1974)

HAMABE, M., T. SATO, AND T. KAWAKAMI. 1974. A preliminary note on the ecology of copulation of the Ommastrephid squid *Illex illecebrosus illecebrosus* Lesueur in the northwest Atlantic. Tokaiku Suisan Kenkyusho Kenkyu Hokoku (78): 97–105. (Transl. from Japanese by Fish. Mar. Ser. Transl. Ser. No. 3320, 1974)

STODOLNIK, L., AND T. DABROWSKI. 1974. Research on cholesterol variations during storage of cod liver and cod

Traductions

Les traductions dont il est question ci-après ont été préparées par ou pour le Service des pêches et des sciences de la mer, à des fins de recherche. On peut en obtenir des exemplaires, à 10 cents la page et à un prix minimum de un dollar, aux Services de traduction, Institut canadien de l'information scientifique et technologique, Conseil national de recherches du Canada, Ottawa (Ontario), K1A 0S2. Le Bureau du rédacteur peut en général fournir des renseignements sur ces traductions.

liver oil. Z. Lebens. -Unters. -Forsch. 154: 84–88. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3321, 1974)

ZUEV, G. V., AND K. N. NESIS. 1971. Squids and bionics, Chapt. 5: 84–90. *In* Squids — biology and fishery. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3322, 1974)

VOVK, A. N., AND C. M. NIGMATULLIN. 1972. Behavior of the flying squid *Ommastrephes pteropus* Steenstrup, 1855 in an illuminated area. Tr. Atl. Nauchno-Issled. Inst. Rybn. Khoz. Okeanogr. (42): 196–197. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3323 1974)

HAYASHI, K., S. KIDA, K. KATO, AND M. YAMADA. 1974. Component fatty acids of acetone-soluble lipids of 17 species of marine benthic algae. Nippon Suisan Gakkaishi 40: 609–617. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3324, 1974)

NESIS, K. N. 1973. Deep-water inhabitants (of the lower sublittoral and bathyal) which do not rise to the surface — Phylum Rossia. Zoology of Invertebrates — Commercial Molluscs 2: 22–23. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3325, 1974)

BJØRNEREM, M. 1974. Capital cost calculations and operating estimates for a fish farming installation. Fiskeridir. Smaskrifter (2): 3–31. (Transl. from Norwegian by Fish. Mar. Serv. Transl. Ser. No. 3326, 1975)

The Fifteenth Supplement to List of Articles in the Translation Series No. 2826–3325, with Indexes to Authors and Translators, is now available from the Department of the Environment, Fisheries and Marine Service, Office of the Editor, 116 Lisgar St., Ottawa, Ont. K1A 0H3.

Le quinzième supplément de la liste des articles dans la série des traductions n° 2826–3325, avec index d'auteurs et de traducteurs, est maintenant disponible au Ministère de l'Environnement, Service des pêches et des sciences de la mer, Bureau du Directeur de la publication, 116 rue Lisgar, Ottawa, Ontario K1A 0H3.

Marine Sciences Directorate Publications

The following publications are available from Information Canada, 171 Slater St., Ottawa, Ont. K1A 0S9 *or* the Canadian Hydrographic Service, 615 Booth St., Ottawa, Ont. K1A 0E6. Cheques or money orders should be made payable to the Receiver General of Canada.

Water Levels, Volume 1 — Inland, 203 p. 1973, Cat. No.: En 35-305/1973-1. $1.00

Water Levels, Volume 2 — Tidal (Part 1), 299 p. 1973, Cat. No.: En 35-305/1973-2-1. $1.00

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Water Levels, Volume 2 — Tidal (Part 2), 163 p. 1973, Cat. No.: En 35-305/1973-2-2. $1.00

Water Levels, Volume 3 — Temporary Gauges, 194 p. 1973, Cat. No.: En 35-305/1973-3. $1.00

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ADDITIONAL PUBLICATIONS / PUBLICATIONS ADDITIONNELLES

The Service also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at establishments located at: Ottawa, Ont.; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

Le Service publie également une série de *Bulletins*, chacun traitant sur un sujet spécifique. Les publications de l'ORP sont vendues par Information Canada à Ottawa. Les remises, payables d'avance en monnaie canadienne, doivent être établies à l'ordre du Receveur général du Canada. Les *Bulletins* sont aussi en vente chez votre libraire et dans les librairies d'Information Canada.

Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages aux établissements situés à Ottawa (Ont.), à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

Journal of the Fisheries Research Board of Canada

Guide for Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

Content

We invite submission of manuscripts in the broad field of fisheries management, ocean science, and aquatic environments relevant to Canada.

Articles: Reports of research studies of broad scope that are original contributions to science.

Notes: Differ from papers on the basis of scope or completeness, rather than quality of research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on methodology or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as articles except that they have no centered headings and should occupy no more than four printed pages.

Reviews: Detailed articles that are critical appraisals of broad areas of investigation, particularly in fields that have shown rapid development in recent years.

Perspectives: Essays of opinion or hypothesis on fisheries, ocean science, aquatic environments, or related topics that are of concern to the professional and layman. References are desirable; informal style is welcomed.

Letters: Opinion on topics recently under discussion in the *Journal*; preferred maximum length, 500 words. The Editor reserves the right to shorten letters and to make other changes to accord with the style of the *Journal*.

Editorial Practices

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970) Special Publication No. 6 of the American Fisheries Society.

Series titles should be avoided. An *abstract* is required for all manuscripts, and should state concisely, in less than 250 words, what was done, the essentials of the new knowledge, and the main conclusions. Abstracts are published in English and French; those submitted in one language only are translated by the *Journal*.

The Editor normally submits each manuscript to at least two *referees*. Contributors are encouraged to suggest at least four names to be added to the list from which referees may be selected.

Illustrations should be submitted in triplicate as photographs (*glossy prints*) no larger than a standard page and must accompany the manuscript. Prints should not be mounted, stapled, or clipped. Symbols, letters, and numerals must be neat, and at least 1.5 mm high when the illustration is reduced to the appropriate size. Drawings should, when possible, be grouped to facilitate comparisons.

Proofs are sent to the author, who is responsible for correcting only errors in typesetting. *Alterations to the content must be avoided.*

Fifty *reprints* are supplied free. Additional reprints may be purchased at the time of publication (an order form is sent out with the galley proof). All orders should be sent to the Printing Department, University of Toronto Press, 5201 Dufferin Street, Downsview, Ont. M3H 5T8. Invoices relating to the reprints will be sent out by the Printer. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the research?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

Journal de l'office des recherches sur les pêcheries du Canada

Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Sujets

Nous désirons reçevoir des manuscrits s'insérant dans le vaste domaine de la gestion des pêches, des sciences de la mer et des milieux aquatiques au Canada.

Articles: Comptes rendus d'études d'une portée générale, constituant des contributions inédites à la science.

Notes: Se distinguent des articles par la portée et le degré d'achèvement plutôt que par la qualité des recherches. Elles peuvent traiter de problèmes particuliers et bien définis; de travaux qui sont en grande partie confirmatoires; de méthodologie ou de techniques de recherche; de progrès de la connaissance découlant d'études plus vastes; de développements dans des projets abandonnés; ou de découvertes préliminaires qui méritent d'être publiées avant la solution de problèmes à l'étude. Les notes peuvent être présentées d'une façon aussi souple que les articles, mais elles ne doivent pas porter d'en-têtes centrés ni occuper plus de quatre pages.

Critiques: Articles détaillés constituant une appréciation critique de vastes champs de recherches, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives: Essais de l'opinion ou de l'hypothèse sur les pêches, les sciences de la mer, les milieux aquatiques, ou sur des sujets connexes qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

Lettres: Opinions sur des sujets exposés dans le *Journal*. Longueur maximale préférable: 500 mots. Le directeur se réserve le droit de raccourcir les lettres et de faire d'autres modifications dans le style du *Journal*.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Il faut éviter les *articles en série*. Un *résumé* doit accompagner chaque texte et doit exposer brièvement (moins de 250 mots) le travail accompli, les points essentiels des connaissances acquises et les principales conclusions. Les résumés paraissent en anglais et en français, le *Journal* veillant à la traduction dans le cas des présentations unilingues.

Le directeur soumet d'ordinaire chaque texte à l'approbation de deux *arbitres* ou plus. Nous encourageons les collaborateurs à suggérer au moins quatre personnes dont les noms pourraient figurer sur la liste des arbitres.

Les *illustrations* doivent être sous forme de photos (*sur papier glacé*), ne pas dépasser les dimensions d'une page normale et accompagner le texte. Elles ne doivent pas être montées, coupées ni brochées. Les symboles, lettres ou chiffres doivent se détacher avec précision et avoir au moins 1,5 mm de hauter lorsque l'illustration est réduite à la grandeur voulue. Les dessins doivent, autant que possible, être groupés pour faciliter l'étude comparative.

L'auteur doit revoir les *épreuves* qu'on lui remet et en corriger seulement les erreurs typographiques. *Il doit éviter de changer le contenu du texte.*

Cinquante *tirés à part* sont fournis gratuitement. Les tirés à part peuvent être achetés au moment de la publication. Un bon de commande à cet effet accompagne les épreuves; il doit être retourné en même temps que celles-ci, non pas au bureau de la rédaction, mais au Printing Department, University of Toronto Press, 5201 Dufferin Street, Downsview, Ont. M3H 5T8, qui se chargera de la facturer. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

Listes de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphs son-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

Journal of the Fisheries Research Board of Canada

Guide for Typists Preparing Copy

Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and two other clear copies to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C). Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send at least 3 *glossy prints* (no larger than 21.5 × 28 cm) of each illustration with the manuscript. Type the author's name and figure number on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the numeral *one*, and between the capital letter *O* and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

Journal de l'office des recherches sur les pêcheries du Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21,5 × 28 cm (8,5 × 11 pouces), laissant toujours une marge d'au moins 2.5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les miniscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, ne nom et l'affiliation des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et ainsi que deux copies lisibles au bureau du Directeur de la publication.

Tableaux. Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).

Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par les minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Envoyez avec le manuscrit trois photos *sur papier glacé* (21,5 × 28 cm ou moins) pour chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le *Journal* (voir Appendice E). Les noms de revues sont abrégés suivants les *Biological Abstracts*; en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

Environment Energy Contents Monthly

Environment Energy Contents Monthly is a new monthly contents periodical designed to encourage interdisciplinary understanding and communication among scientists, professionals, and students involved in energy and environmental work. The publication presents tables of contents from over 400 U.S. and foreign scientific and professional journals that include articles and papers on environment, ecology, and energy, all in original format. Cost of a one year subscription is $16.00. Additional information is available from Environment Energy Institute, P.O. Box 1450, Portland, Oregon, USA 97207.

Le nouveau périodique mensuel *Environment Energy Contents Monthly* a été créé pour favoriser la compréhension et la communication interdisciplinaires entre scientifiques, professionnels et étudiants du domaine de l'environnement et de l'énergie. La publication contient, dans leur présentation initiale, les tables des matières de plus de 400 revues scientifiques et professionnelles des États-Unis et d'ailleurs, ce qui comprend des articles et des exposés sur l'environnement, l'écologie et l'énergie. L'abonnement pour un an est de $16. Pour plus de renseignements, écrire à: Environment Energy Institute, P.O. Box 1450, Portland, Oregon, USA 97207.

Recent Bulletins/Récents Bulletins

183 Russian–English dictionary for students of fisheries and aquatic biology
W. E. RICKER
(1973, 428 p., cat. no. Fs 94-183; Canada: $7.00, Other countries/Autres pays: $8.40)

184 Freshwater fishes of Canada
W. B. SCOTT AND E. J. CROSSMAN
(1973, 966 p., cat. no. Fs 94-184; Canada: $9.75, Other countries: $11.70)

184F Poissons d'eau douce du Canada
W. B. SCOTT ET E. J. CROSSMAN
(1974, 1026 p., n° de cat. Fs 94-184F; Canada: $9.75, Autres pays: $11.70)

185 Benthic errantiate polychaetes of British Columbia and Washington
KARL BANSE AND KATHARINE D. HOBSON
(1974, 111 p., cat. no. Fs 94-185; Canada: $4.50, Other countries/Autres pays: $5.40)

186 The capelin (*Mallotus villosus*): biology, distribution, exploitation, utilization, and composition
P. M. JANGAARD
(1974, 70 p., cat. no. Fs 94-186; Canada: $3.00, Other countries/Autres pays: $3.60)

186F Le capelan (*Mallotus villosus*): biologie, distribution, exploitation, utilisation et composition
P. M. JANGAARD
(1975, 77 p., n° de cat. Fs 94-186F; Canada: $3.00; Autres pays $3.60)

187 The lobster fishery of the Maritime Provinces: economic effects of regulations
A. GORDON DEWOLF
(1974, 59 p., cat. no. Fs 94-187; Canada: $3.00, Other countries/Autres pays: $3.60)

188 Aquaculture in Canada — the practice and the promise
H. R. MACCRIMMON, J. E. STEWART, AND J. R. BRETT
(1974, 84 p., cat. no. Fs 94-188; Canada: $3.75, Other countries/Autres pays: $4.50)

189 Treatment of fish processing plant wastewater
F. G. CLAGGETT AND J. WONG
(1974, 18 p., cat. no. Fs 94-189; Canada: $2.00, Other countries/Autres pays: $2.40)

190 Hydrodynamics and energetics of fish propulsion
PAUL W. WEBB
(1975, 159 p., cat. no. Fs 94-190; Canada: $5.00, Other countries/Autres pays: $6.00)

191 Computation and interpretation of biological statistics of fish populations
W. E. RICKER
(1975, 382 p., cat. no. Fs 94-191; Canada: $8.00, Other countries/Autres pays: $9.60)

192 Catalogue and synopsis of *Caligus*, a genus of Copepoda (Crustacea) parasitic on fishes
L. MARGOLIS, Z. KABATA, AND R. R. PARKER
(1975, 117 p., cat. no. Fs 94-192; Canada: $5.00, Other countries/Autres pays: $6.00)

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Journal of the Fisheries Research Board of Canada

Journal de l'office des recherches sur les pêcheries du Canada

JFRBAK32(5)587–728(1975)

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Diets of juvenile Atlantic menhaden (*Brevoortia tyrannus*) in three estuarine habitats as determined from fatty acid composition of gut contents **H. P. Jeffries** 587–592

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Toxaphene effects on growth and development of brook trout (*Salvelinus fontinalis*) **P. M. Mehrle and F. L. Mayer Jr.** 609–613

Chemical changes during the seasonal cycle of growth and decay in eelgrass (*Zostera marina*) on the Atlantic coast of Canada **P. G. Harrison and K. H. Mann** 615–621

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Pectoral fin and opercular movements of Atlantic salmon (*Salmo salar*) alevins **R. H. Peterson** 643–647

New coccidia from the spiny dogfish shark (*Squalus acanthias*) and great sculpin (*Myoxocephalus polyacanthocephalus*) **P. R. Fitzgerald** 649–651

Meristic differences in spawning populations of American shad, *Alosa sapidissima*: evidence for homing to tributaries in the St. John River, New Brunswick **J. E. Carscadden and W. C. Leggett** 653–660

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